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The Received Power Patterns due to Microwave Paraboloidal Reflector

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A detailed mathematical theory has been developed for the calculation of power patterns in the Fresnel and quasi-Fraunhofer regions for a microwave paraboloidal reflector with a dipole source at its focus. Two different methods for evaluating the integral in the expression for received power are described. The theory has been experimentally verified for a simplified case of the power received along the axis of the paraboloid in the Fresnel and quasi-Fraunhofer regions.

A METALLIC reflecting surface excited by a small radiating source is generally used to transmit microwaves in the form of a directional beam. For the maximum directivity of the antenna system, the reflector shape is usually paraboloid with the feed located at its focus and directed towards the reflecting surface. It is now known that at any point away from the aerial system on the horizontal plane passing through the apex in the direction of the beam, the power received is mainly due to the waves reflected from the apex of the paraboloid and due to the diffracted rays from the periphery of the paraboloid. Due to the phase difference between these waves, it is found that as one goes away from the aperture plane of the paraboloid, there will be maxima and minima in the power received up to a distance of $D^2/4\lambda$ where the phase difference between the waves is π-radians, and D represents the diameter of the aperture and λ is the operating wavelength. This region of variable intensity of the received wave is known as the Fresnel region. Beyond this region, however, the phase difference gradually decreases and reduces to $\pi/8$-radians at a distance of $2D^2/\lambda$, the region beyond which is known as the Fraunhofer region. The space between $2D^2/\lambda$ and $D^2/4\lambda$ is called the quasi-Fraunhofer region[1].

The method of calculation of power received in the Fraunhofer region has been developed by Silver[2], but the expressions for the received power in the Fresnel and quasi-Fraunhofer regions have not so far been worked out. The present communication deals with a detailed mathematical analysis for the calculation of power patterns in the Fresnel region which can also be extended to the quasi-Fraunhofer region. The above analysis has been partly verified by the experimental study of the power received along the axis of a microwave paraboloidal reflector in the Fresnel and quasi-Fraunhofer regions.

Theoretical considerations

Kirchoff's mathematical formula[3] has been applied for the development of the theory of formation of

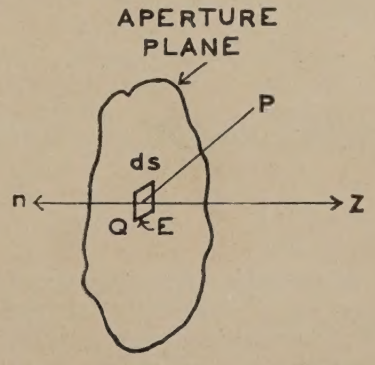

FIG. 1 — FIELD AT AN EXTERNAL POINT P DUE TO AN INFINITESIMAL AREA ON THE APERTURE PLANE

the power patterns due to a microwave paraboloidal reflector with a dipole aerial as the feed located at its focus when the size of the dipole is small compared to the dimensions of the aperture of the paraboloid.

Let the electric field at the infinitesimally small area dS on the plane of the aperture, as shown in Fig. 1, be denoted by E. The field at a point P away from the aperture according to the Kirchoff's formula is then given by

$$E_P = \frac{1}{4\pi} \oint_S \left[\frac{e^{-jkr}}{r} \, grad\, E - E \, grad \frac{e^{-jkr}}{r} \right] dS \quad \ldots \quad (1)$$

where E_P is the field due to the total effect of all the infinitesimal elements on the surface S of the aperture, $k = 2\pi/\lambda$, and λ is the wavelength.

As E is a constant scalar field over the aperture, we may write

$$grad\, E = \frac{\partial E}{\partial n} \quad \ldots \quad (2)$$

where n is the unit vector, normal to the surface of the aperture plane in the direction opposite to that of the Z-axis.

If the distance of the point P (r, θ) is sufficiently large compared to the wavelength of transmission, we get after neglecting higher powers of $1/r$,

$$grad \frac{e^{-jkr}}{r} \doteq -\frac{jk}{r} e^{-jkr} \cos\theta \quad \ldots \quad (3)$$

Therefore, from equation (1) we get

$$E_P = \frac{1}{4\pi} \oint_S \frac{e^{-jkr}}{r} \left(\frac{\partial E}{\partial n} + jkE \cos\theta \right) dS \quad \ldots \quad (4)$$

Now the varying field can be expressed by

$$E = E_o \, e^{j(\omega t - kz)} \quad \ldots \quad (5)$$

where ω is the angular frequency and z is the distance from the centre of the aperture to the point of observation in the direction of propagation of the wave along the Z-axis.

Therefore, from the above equation, we get after differentiation,

$$\frac{\partial E}{\partial n} = -\frac{\partial E}{\partial z} = jkE \quad \ldots \quad (6)$$

Substituting the above value of $\frac{\partial E}{\partial n}$ in equation (4) we get,

$$E_P = \frac{jkE}{4\pi} \oint_S \frac{e^{-jkr}}{r} (1 + \cos\theta) dS \quad \ldots \quad (7)$$

Let us now consider a circular aperture of radius a, as shown in Fig. 2. A point Q on the small surface dS on the circular aperture, and an external point P are represented as $(x_1, y_1, 0)$ and (x, y, z) respectively in rectangular co-ordinates measured with respect to the centre O of the aperture. If the distance between the points P and Q be denoted by r, then

$$r^2 = (x - x_1)^2 + (y - y_1)^2 + z^2 \quad \ldots \quad (8)$$

As the measurements are made in the horizontal plane, $y = 0$ and equation (8) reduces to

$$r = z \left[1 + \frac{(x - x_1)^2 + y_1^2}{z^2} \right]^{\frac{1}{2}} \quad \ldots \quad (9)$$

For the observations in the Fresnel region, which extends up to 5 m. from the centre of the aperture plane of the paraboloid for the wavelength used in the present investigation, it will be seen that z^2 will be large compared to $(x - x_1)^2 + y_1^2$ even up to a distance of 2 m. from the aperture and thus in the expansion of the expression under square root in equation (9). The higher powers of $\left[\frac{(x - x_1)^2 + y_1^2}{z^2} \right]$ may be neglected.

Thus equation (9) may then be written as

$$r = z + \frac{(x - x_1)^2 + y_1^2}{2z} \quad \ldots \quad (10)$$

Expressing P and Q in polar co-ordinates as (r_1, θ) and (ρ, ϕ) respectively, we have

$$r = r_1 \cos\theta + \frac{r_1}{2} \frac{\sin^2\theta}{\cos\theta} + \frac{\rho^2}{2r_1 \cos\theta} - \rho \tan\theta \cos\phi \quad \ldots \quad (11)$$

Substituting the above value of r in equation (7) and simplifying, we get

$$E_P = \frac{jk}{2r_1} \cdot E(1 + \cos\theta) e^{-jk\left(r_1 \cos\theta + \frac{r_1}{2} \frac{\sin^2\theta}{\cos\theta}\right)} \int_o^a e^{-j\left(\frac{k\rho^2}{2r_1 \cos\theta}\right)} \cdot J_o(k\rho \tan\theta)\, \rho \, d\rho \quad \ldots \quad (12)$$

It may be mentioned that the integral in equation (12) is fairly complicated. However, theoretically the power received on the axis of the paraboloidal reflector can be calculated easily by substituting $\theta = 0$

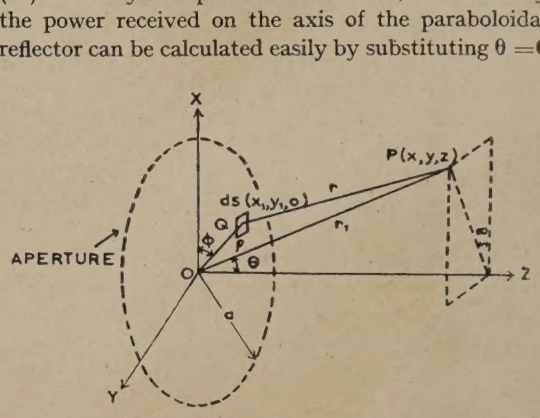

FIG. 2 — FIELD AT A POINT P DUE TO THE CIRCULAR APERTURE OF THE PARABOLOID

in equation (12) which can then be simplified and written as

$$E_P = -E\, e^{-jkr_1}\left[e^{-\frac{jka^2}{2r_1}} - 1\right] \quad \ldots\ldots (13)$$

The real value of E_P will be given by

$$|E_P| = 2E \sin\frac{\pi a^2}{2\lambda r_1} \quad \ldots\ldots (14)$$

The power along the axis can, therefore, be expressed by

$$W_{axis} = K\left[4E^2 \sin^2\left(\frac{\pi a^2}{2\lambda r_1}\right)\right] \quad \ldots\ldots (15)$$

where K is the constant of proportionality.

The above expression has been verified experimentally by taking observations of received power along the axis, the method of which has been described later.

Now in order to evaluate the integral in equation (12), it may be simplified by putting

$$\eta = k \tan\theta,\ \xi = \frac{k}{2r_1\cos\theta} \text{ and } t = \frac{\rho}{a}$$

Thus we get,

$$E_P = \frac{jka^2}{2r_1} E(1+\cos\theta)\, e^{-jkr_1\cos\theta} \cdot e^{-\frac{j\eta^2}{4\xi}} \int_0^1 e^{-j\xi t^2a^2} J_o(\eta at)t.dt \quad \ldots (16)$$

Evaluation of the integral

The integral in equation (16) can now be evaluated by the following two methods:

1. Let the integral be denoted by

$$g(t) = \int_0^1 e^{-j\xi t^2a^2} J_o(\eta at)t.dt \quad \ldots\ldots (17)$$

Using the expansion of $J_o(\eta at)$, we get

$$g(t) = \sum_{m=0}^{\infty} (-1)^m \frac{(\frac{1}{2}\eta a)^{2m}}{(\underline{|m})^2} \int_0^1 t^{2m+1} . e^{-j\xi t^2a^2} . dt \quad \ldots (18)$$

The integrand in the above equation can be expressed as derivatives of $g_o(t)$, which is the value of $g(t)$ when $\eta = 0$

Thus

$$g_o(t) = \int_0^1 e^{-j\xi t^2a^2} t.dt = \frac{j}{2\xi a^2}\left(e^{-j\xi a^2} - 1\right) \quad \ldots\ldots (19)$$

and its derivatives are given by

$$\frac{d^k}{d\xi^k} g_o(t) = (-j)^k a^{2k} \int_0^1 t^{2k+1} e^{-j\xi t^2a^2} dt \quad \ldots\ldots (20)$$

Putting $k = m$ and substituting the value of the integrand from equation (20), equation (18) reduces to

$$g(t) = \sum_{m=0}^{\infty} (-1)^m \frac{(\frac{1}{2}\eta a)^{2m}}{(\underline{|m})^2} \cdot \frac{1}{(-j)^m a^{2m}} \frac{d^m}{d\xi^m} g_o(t) \quad \ldots (21)$$

Then from the above value of $g(t)$, E_P can be determined from equation (16).

2. The second method of solving equation (16) is by making the following substitutions, namely

$$\eta = \frac{p\chi}{a} \text{ and } \xi = \frac{p}{2a^2}$$

so that

$$E_P = \frac{k}{4\xi r_1} E(1+\cos\theta)\, e^{-jkr_1\cos\theta} . \phi(t) \quad \ldots\ldots (22)$$

where

$$\phi(t) = je^{-j\left(\frac{p\chi^2}{2}\right)} . p\int_0^1 e^{-j\left(\frac{p}{2}\right)t^2} J_o(p\chi t)t.\, dt$$

The value of $\phi(t)$ can be determined by forming a differential equation of the type

$$\phi'(t)e^{-j\left(\frac{p\chi^2}{2}\right)} - pJ_1(p\chi)e^{-j\left(\frac{p}{2}\right)} = 0 \quad \ldots\ldots (23)$$

It may be mentioned that although it is difficult to solve the above equation, analogue computer may be used as indicated by Jakes[4] for solving equations of similar type.

Experimental procedure

A folded dipole was excited by a magnetron oscillator generating a wavelength of 20 cm. with a matched coaxial cable and a small truncated paraboloid of focal length 4·5 cm. was placed behind it to direct the waves towards the paraboloid. The dipole was kept at the focus of the paraboloidal reflector of focal length 65 cm. made of fine steel wire netting with clearances of dimensional area 0·086 sq. cm. and with an average diameter of the wires 0·02 cm. The grid structure in the design of the paraboloids reduces the weight and the effects of wind on them and maintains the visibility through the system. The dimension of the aperture is determined from the following considerations.

It is now known[5] that if the aperture of the paraboloid is made too large, the total radiation is reduced. On the other hand, if the diameter of the aperture is too small, pronounced diffraction effects from the edges of the paraboloid result in broadening the half-power beam-width angle of the radiated beam. The

diameter of the aperture was, therefore, kept as 10λ in order to minimize the loss of radiation. For increasing the intensity of radiation after reflection from the paraboloidal surface, the design of the paraboloid was so made that the focal length was slightly greater than the distance between the apex and the centre of the aperture of the paraboloid.

The receiver consisted of a germanium crystal detector placed at the focus of another small paraboloidal reflector with aperture diameter of 10 cm. and focal length 5 cm. so that the scattered field due to the interaction between the transmitting and the receiving aerial systems may be reduced. As the germanium crystal detects the signal voltage on the basis of square law detection at low power, the rectified current is proportional to the square of the applied voltage. A d.c. microammeter was connected across the crystal with long twisted flexible wire and was kept away from the crystal to reduce the stray capacity effect. Thus the power received was directly proportional to the deflection of the microammeter. The observations were made in the horizontal plane passing through the apex of the paraboloid.

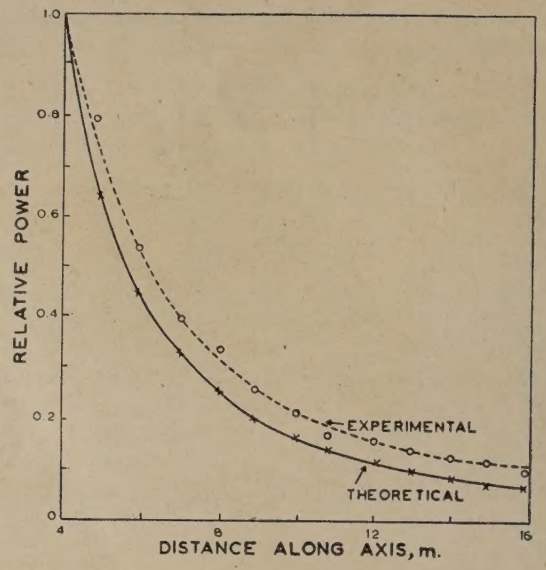

FIG. 4 — VARIATION OF POWER RECEIVED FROM A MICROWAVE PARABOLOIDAL REFLECTOR ALONG ITS AXIS

Results

The experimental angular variation of received relative power for various distances from the aperture plane of the paraboloid in the Fresnel and quasi-Fraunhofer regions is shown in Cartesian co-ordinates in Fig. 3. For drawing the power patterns, the Cartesian co-ordinate system is preferred over the polar co-ordinates due to the fact that a detailed study is possible only in Cartesian co-ordinates for any small variation of received power corresponding to the different angles for various distances from the aperture plane. The curves for 3 and 5 m. in Fig. 3 represent the variation of relative power in the Fresnel region and the rest of the curves are for the quasi-Fraunhofer region.

For the verification of the theoretical analysis, the variation of the power received along the axis of the paraboloid was determined experimentally, the curve for which is drawn along with that obtained from theoretically calculated values of received power from equation (15) and the curves are shown in Fig. 4.

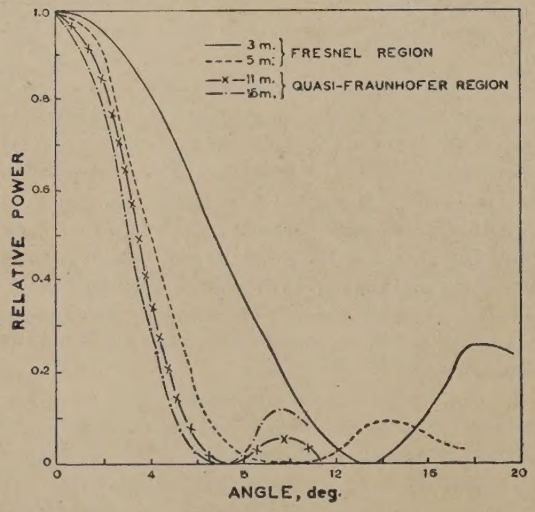

FIG. 3 — ANGULAR VARIATION OF RECEIVED RELATIVE POWER IN THE FRESNEL AND QUASI-FRAUNHOFER REGIONS

Discussion

It will be observed from the curves in Fig. 3 that for distances below 15 m. there is the indication of the secondary lobes at about 9° and this angle slowly increases as the receiving point approaches the Fresnel region. The energy in the secondary lobe gradually increases as the distance between the receiving point and the centre of the aperture decreases. It may also be observed that the half-power beam-width angle of the main lobe in the Fresnel region is large compared to that in the quasi-Fraunhofer region.

As it is difficult to obtain the power patterns in the Fresnel and quasi-Fraunhofer regions from the mathematical analysis given earlier, the direct verification of the theory for complete analysis is not possible. The theory has, therefore, been experimentally verified for a simplified case for the power received along the axis of the paraboloid in the above regions. It will be seen from Fig. 4 that the theoretical and

experimental curves agree almost closely with one another within the limits of experimental error.

Acknowledgement

The authors have pleasure in recording their grateful thanks to Dr S. S. Banerjee for his continued guidance and discussions during the course of the above investigations. They also wish to express their sincere thanks to Shri M. Sen Gupta, Principal, for his helpful interest. Thanks are also due to the Government of Uttar Pradesh and to the Council of Scientific & Industrial Research, New Delhi, for financial assistance.

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Dielectric Dispersion of Polar Liquids: Part III—Asymmetric Dispersion in Glycerol

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The dielectric dispersion of glycerol has been studied at the laboratory temperature (28°C.) in the microwave and ultra-high frequency regions. It is observed that the behaviour of glycerol could be well represented by an equation proposed by Davidson and Cole, namely $\epsilon^*=\epsilon_\infty+(\epsilon_s-\epsilon_\infty)/(1+j\omega\tau')^b$ where $b=0{\cdot}592$ and $\tau'=10{\cdot}04\times10^{-10}$ sec. A correlation has been attempted between Davidson and Cole's measurements and the measurements carried out by the author by a study of the variation of τ' with temperature. The results point out that the skewed arc locus is a fair representation of the data over a wide range of temperatures (28° to −70°C.). The temperature dependence of relaxation time indicates that low temperatures may not play an important role in the asymmetric frequency dependence of dispersion.

MIZUSHIMA[1] and Morgan[2] studied the dielectric properties of glycerol at different frequencies and temperatures and the data were fitted only qualitatively into the simple Debye equation. These data, however, were not extensive enough for a more satisfactory function to be uniquely determined. Later incidental measurements on glycerol in the supercooled liquid state by Davidson and Cole[3,4] showed that the dielectric relaxation process exhibits simple and characteristic but unexpected properties, which are not readily explained in terms of any simple picture of molecular association or bonding of hydroxyl groups. Whereas a large number of dielectrics give data consistent with the usual symmetric dispersion, glycerol and propylene glycol exhibited, according to the measurements of Davidson and Cole, at all the temperatures investigated, an asymmetric frequency dependence over a wide temperature and frequency range (−40° to −70°C. and 20 c/s. to 5 Mc/s.). As far as comparison could be made, the results of Davidson and Cole on glycerol were in fair agreement with those obtained by Morgan.

Davidson and Cole interpreted the results by an empirical expression for the complex dielectric constant as a function of frequency of the form†

$$\epsilon^*=\epsilon'-j\epsilon''=\epsilon_\infty+\frac{\epsilon_s-\epsilon_\infty}{(1+j\omega\tau')^b} \quad \ldots\ldots (1)$$

where the parameter b may assume a value between 0 and 1 and τ' is the relaxation time and the other

†In this expression b is used instead of β used by Davidson and Cole in order to avoid confusion with the β used in Kirkwood equation quoted in Part II of this series.

symbols have the usual significance. It may be noted that $\tau' = \frac{1}{2\pi f_c}$ is different from τ obtained from a symmetric dispersion formula[5] (Part II of this series) and the frequency f_c is lower than the frequency for which ϵ'' is a maximum and corresponds to the point on the complex plane locus for which $\theta = b\pi/4$. The shape of the complex plane locus is essentially asymmetric. This behaviour of glycerol was described by Davidson and Cole on the basis that the observed relaxation is the sum of two processes: (i) a process satisfying equation (1) and (ii) a process involving a smaller contribution at very high frequencies.

After the publication of Davidson and Cole's results, other interpretations have been put forward concerning the significance of the peculiar dielectric behaviour of glycerol. Magat and co-workers[6] have stated that this behaviour is analogous to the distinct secondary high frequency dispersion found in a number of aliphatic alcohols. But Cole[7] has shown that this analogy is not appropriate.

Böttcher[8] suggested that in the case of the broader dispersion observed for frequencies $\omega > \omega_m$, the dielectric relaxation process may be explained in terms of a number of different processes rather than a single process combining the different processes. Poley[9] proposed that the behaviour of glycerol could be explained by a Debye function characterized by a long relaxation time, a second symmetric arc dispersion function and a high frequency dispersion similar to the one obtained by Davidson and Cole. Poley has also drawn attention to the fact that by an analysis of the data suggested by him, a continuous distribution of relaxation times could be maintained while Davidson and Cole's analysis gives rise to a dubious distribution function of relaxation times. Cole, however, has shown that the complex plane locus of the residual dispersion and loss would result in a curious shape, quite out of conformity with experimental points. Cole has also stated that the essential point to be considered is how well the equation suggested by him fits the data and that, when the suggested equation is adequate, the form of distribution function has to be derived from it.

Similar skewed types of arc dispersions represented by equation (1) have since been noticed in certain liquids[10,11] other than glycerol and propylene glycol, in which it was initially found, whereas in the case of certain other liquids, viz. 1-propanol and related alcohols, ethanol 2-propanol[12] and liquid butyl alcohols[13], two or three distinct regions of dispersion have been found all showing the exponential temperature dependence which is characteristic of dielectric relaxation process. It seemed that hydrogen bonding played an important role in dielectric dispersion and that asymmetric distribution of relaxation times might in some way be related to the extensive cross-linking in polyhydroxy compounds through hydrogen bonding. However, later observations by Denney[10] showed the existence of the skewed arc locus in the complex plane similar to that found in glycerol, also in some liquid alkyl halides (namely isobutyl chloride and bromide and isoamyl bromide) which have little or no hydrogen bonding. This unexpected result leads to the need for considering several other factors such as molecular complexity at low temperatures, short-range intermolecular forces, etc., other than hydrogen bonding, as influencing the above-mentioned characteristics in alcohols and glycols. On the other hand, the further results obtained by Winslow *et al.*[11] on certain viscous polar liquids seem to lead to the conclusion arrived at by these authors that the departure from the Debye behaviour in dielectrics might be due to some property having relatively little to do with dielectric properties or molecular structure. It was supposed that this critical property is the rate of attainment of equilibrium energy distribution among the various degrees of freedom of the system.

A study of the observations detailed above has stimulated the interest of the author in investigations on dielectric dispersion in a variety of polar liquids, the results of which have been described in earlier parts of this series[5,14]. The present paper reports certain experiments on glycerol, the first liquid in which asymmetric dispersion was observed. The work of Davidson and Cole on glycerol, although extensive enough, had to be carried out in a temperature range at which the liquid is in a supercooled state, on account of the availability of the required wide and continuous range of frequencies in the kilocycle and lower frequency regions. The present work is an extension of Davidson and Cole's investigation into the region of ultra-high and microwave frequencies at room temperature. The purpose is essentially to study whether the same skewed type of arc would result even at higher temperatures and to study the temperature dependence of relaxation in glycerol, over a wider range of temperatures.

A series of G.R. oscillators providing a continuous range of frequencies from 300 to 2000 Mc/s., a Q-meter giving a signal in the 20 Mc/s. range and three klystron sources of 4, 3 and 2 cm. facilitated the measurements carried out in the present study. Some observations were taken at 1 cm. wavelength also with a 2K33 klystron, but these were not considered sufficiently reliable due to difficulties experienced in working with a dielectric cell of the small dimensions needed for work at 1 cm.

The dielectric constant ϵ' and loss factor ϵ'' at different frequencies have been measured using waveguide components for centimetre region[5,14] and coaxial line[15] with G.R. type oscillators. Details of the experimental arrangements and method of calculation of ϵ' and ϵ'' have already been given elsewhere[14,15].

Results and discussion

The observed and calculated dispersion values at different frequencies are presented in Table 1. In Fig. 1 the values of ϵ'' are plotted against those of ϵ'. Assuming equation (1) to be valid, the parameters ϵ_s, ϵ_∞, b and τ' could be obtained from the observations in the following manner, adopting the methods of Davidson and Cole and of Denney[10].

Separating the real and imaginary parts of equation (1) we have

$$\left.\begin{aligned}\epsilon'-\epsilon_\infty&=(\epsilon_s-\epsilon_\infty)(\cos\Psi)^b\cos b\Psi\\ \epsilon''&=(\epsilon_s-\epsilon_\infty)(\cos\Psi)^b\sin b\Psi\end{aligned}\right\} \quad \ldots\ldots (2)$$

where $\omega\tau'=\tan\Psi$. Expressing in polar co-ordinates (r, θ) we obtain the equations

$$[(\epsilon'-\epsilon_\infty)^2+\epsilon''^2]^{\frac{1}{2}}=r=(\epsilon_s-\epsilon_\infty)\left(\cos\frac{\theta}{b}\right)^b \quad \ldots\ldots (3)$$

$$\omega\tau'=\tan\left(\frac{\theta}{b}\right) \quad \ldots\ldots\ldots\ldots (4)$$

where $$\theta=b\Psi=\tan^{-1}\epsilon''/(\epsilon'-\epsilon_\infty) \quad \ldots\ldots\ldots (5)$$

or $$\epsilon'=[\epsilon''/\tan b\Psi]+\epsilon_\infty \quad \ldots\ldots\ldots\ldots (6)$$

Preliminary values of ϵ_∞ and b were obtained from the locus of the points of ϵ'' and ϵ' in Fig. 1, $b\pi/2$ being the angle between the ϵ' axis and the line asymptotic to the points corresponding to high frequency. Using these preliminary values in equations (5) and (6), a plot of log (tan Ψ) versus log (frequency) is obtained. If the preliminary values are correct, these should give a straight line of slope unity, and the intercept on log (frequency) axis for tan $\Psi = 1$ gives the parameter τ'. In the actual analysis a number of such trial curves between log (tan Ψ) and log (frequency) are plotted and the correct plots are chosen. Three of these plots are shown in Fig. 2. The values of the parameters finally arrived at are shown in Table 1.

TABLE 1 — VALUES OF DIELECTRIC DISPERSION AND DIELECTRIC LOSS FOR GLYCEROL

(*Temp.*, 28°C.)

f Mc/s.	$\epsilon'_{obs.}$	$\epsilon'_{calc.}$	$\epsilon''_{obs.}$	$\epsilon''_{calc.}$	Log tan (θ/b)
15	41·31	41·30	2·11	2·12	−1·024
30	41·18	41·10	4·13	4·10	−0·721
300	22·88	23·13	14·60	14·25	0·276
400	18·93	19·60	13·20	13·45	0·402
450	17·85	18·52	12·01	12·90	0·453
500	15·92	15·80	11·20	11·11	0·498
900	12·79	12·99	9·69	9·69	0·754
1000	11·98	12·38	9·02	9·27	0·799
1100	11·74	11·85	8·80	8·84	0·841
1200	11·30	11·40	8·46	8·45	0·879
1300	10·96	11·01	8·14	8·10	0·914
1400	10·57	10·69	7·79	7·81	0·946
1500	10·29	10·36	7·52	7·50	0·978
1600	9·95	10·14	7·16	7·29	1·002
1700	9·78	9·90	7·00	7·06	1·028
1800	9·58	9·68	6·82	6·83	1·045
2000	9·17	9·30	6·40	6·46	1·099
6900	6·42	6·54	3·10	3·24	1·612
9200	5·90	6·01	2·54	2·71	1·758
13800	4·70	5·64	1·35	2·06	1·980

$\epsilon_s = 41{\cdot}9$; $\epsilon_\infty = 4{\cdot}00$; $b = 0{\cdot}592$; $\tau' = 10{\cdot}04\times10^{-10}$ sec.

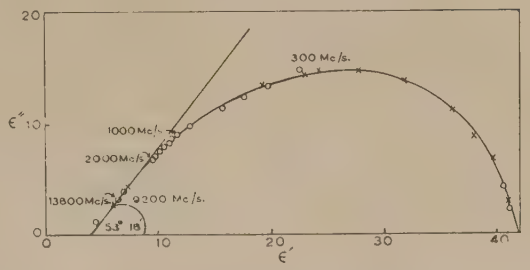

FIG. 1 — COMPLEX PLANE LOCUS OF ϵ'' VERSUS ϵ' FOR GLYCEROL AT 28°C. [Solid line represents theoretical curve while circles represent experimental points]

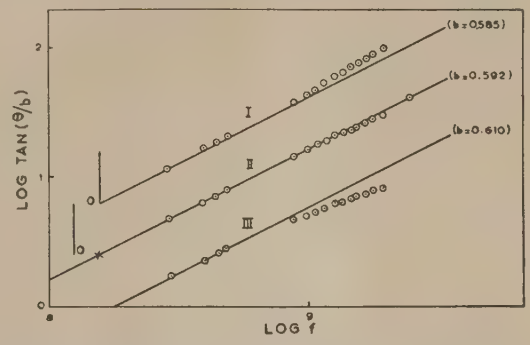

FIG. 2 — EFFECT OF DIFFERENT VALUES OF b ON LINEARITY OF THE PLOT OF LOG (TAN θ/b) VERSUS LOG (FREQUENCY) [To avoid overlapping of the curves, the log (f) axis in the case of curves I and II is shifted upwards as indicated]

As a check on the value of ϵ_∞ obtained by the above procedure a curve is drawn between ϵ' and $\epsilon''/\tan b\Psi$ which, according to equation (6), should be a straight line, with ϵ_∞ equal to the intercept on ϵ' axis. This curve is shown in Fig. 3. The value of ϵ_∞ thus derived is equal to 4·0 consistent with that determined above.

Using the final values of the parameters the complex plane locus of ϵ'' versus ϵ' is calculated. This is shown as a solid line in Fig. 1, while the circles are the experimental points. The validity of Davidson and Cole's equation as representing the dielectric behaviour of glycerol at the temperature of observation is clear. The calculated values of ϵ' and ϵ'' are also shown in Table 1. Agreement with observed

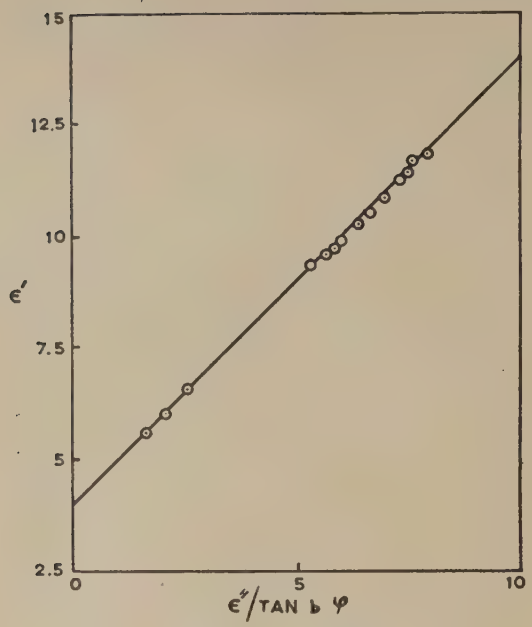

FIG. 3 — VARIATION OF ϵ' WITH ϵ''/TAN $(b\varphi)$

TABLE 2 — RELAXATION PARAMETERS FOR GLYCEROL AT DIFFERENT TEMPERATURES

TEMP. °C.	τ' sec.		b
28·0	$10{\cdot}04\times10^{-10}$	Author's value	0·592
−40·0	$1{\cdot}15\times10^{-5}$	Davidson and Cole's values	0·608
−50·0	$1{\cdot}25\times10^{-4}$		0·603
−60·0	$2{\cdot}50\times10^{-3}$*		0·593
−64·5	$1{\cdot}86\times10^{-2}$*		0·595
−70·0	0·118*		0·566

*Values from transient measurements.

values is within experimental error except at the highest frequency. There may be a second region of dielectric dispersion which is inferred also from the large difference between the value of ϵ_∞ (4·00) and the square of the refractive index ($n^2 = 2{\cdot}16$).

A correlation is attempted between the results of Davidson and Cole at low temperatures and the results obtained in the present investigation at a higher temperature by examining the variation of relaxation times with temperature. The relaxation times are known to vary markedly with temperature in the case of a polar liquid in the manner expected of a chemical rate process. Table 2 gives the consolidated data on the variation of relaxation times with temperature, obtained in the two investigations. The expression

$$\tau' = A e^{B/T-T_\infty}$$

where $T_\infty = 132°K.$, is assumed.

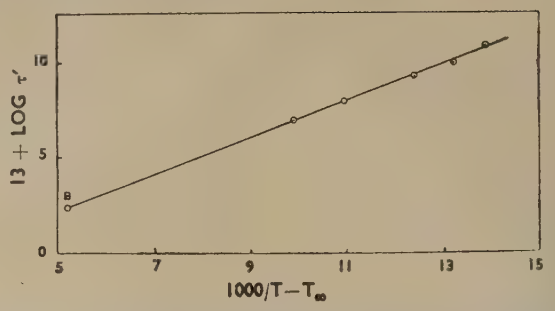

FIG. 4 — LOG (τ') AS A FUNCTION OF RECIPROCAL OF $(T-T_\infty)$ [The point B corresponds to the value of temperature at which the present investigation has been conducted]

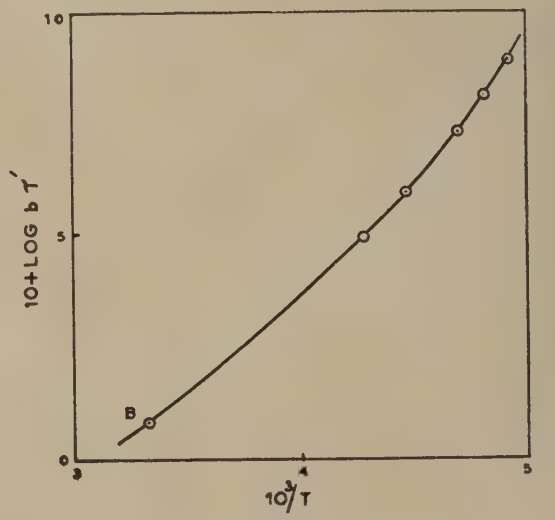

FIG. 5 — TEMPERATURE DEPENDENCE OF THE RELAXATION PARAMETER $b\tau'$

A plot of log τ' versus $10^3/T-T_\infty$ is shown in Fig. 4. The point B on the curve corresponds to the value of the temperature at which observations have been made in the present investigation. The plot is a straight line along which other points corresponding to Davidson and Cole's data also occur.

The temperature dependence of τ' may also be studied by plotting the relaxation parameter $b\tau'$ against temperature. The plot of log $b\tau'$ versus $10^3/T$ is shown in Fig. 5. The form of the curve is similar to the one obtained by Denney in liquid halides.

Acknowledgement

The author is deeply indebted to Prof. K. R. Rao for his invaluable guidance throughout the progress of the work. He is also grateful to the Council of Scientific & Industrial Research for the award of a Junior Research Fellowship.

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Adiabatic Compressibilities of Electrolytic Solutions

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The adiabatic compressibility as a function of concentration has been studied for aqueous solutions of sodium hydroxide, potassium hydroxide, sodium nitrate, potassium nitrate, sodium thiosulphate, lead nitrate and cadmium iodide, using a glass piezometer. From the compressibility data, sound velocities at different concentrations have been calculated for solutions of lead nitrate and cadmium iodide. In both the cases, the calculated velocities show a decrease with increasing concentration, confirming the results obtained by the ultrasonic method. This peculiar behaviour of these solutions has been explained as due to the relatively heavy mass of the metallic ions.

EARLY measurements of adiabatic compressibilities of liquids have been carried out by Tyrer[1], Dakshinamurthi[2], Philip[3] and others, by applying a static compression and noting the change produced in a known volume of the liquid contained in a piezometer. Adiabatic compressibility can also be evaluated from a knowledge of sound velocity. Ultrasonic methods of measuring sound velocity have been used in recent years to evaluate the compressibilities of liquids. Such studies have also been extended to a study of sound velocity and adiabatic compressibility as a function of concentration of electrolytic solutions. Electrolytes, in general, show an increase of sound velocity and a decrease of adiabatic compressibility with increasing concentration. But aqueous solutions of lead nitrate[4] and cadmium iodide[5] are found to behave in a peculiar manner. In both the cases, the sound velocity decreases with increasing concentration. However, the adiabatic compressibilities show a decrease with increasing concentration in accordance with the general behaviour of electrolytes.

The static and sound velocity methods of obtaining compressibility are entirely different in principle and relate to different circumstances under which the liquid is compressed. Hence, it was thought desirable to study the compressibility behaviour of lead nitrate and cadmium iodide by the piezometer method and compare the results with the existing ultrasonic data. Incidentally, a number of other electrolytic solutions have also been investigated. The results of this study have been presented in this paper.

Experimental procedure

The piezometer used in the present investigation is similar to the one used by Tyrer[1] and Dakshinamurthi[2]. The liquid container and the outer jacket have been modified to minimize the dead space of the instrument and facilitate quick filling and withdrawal of the liquid. The piezometer assembly is shown in Fig. 1. The piezometer bulb A, made of pyrex glass, has a volume of 344 ml. To the top end of it is connected the capillary tube C of radius 0·0394 cm. At the free end of the capillary is a glass tube T with a

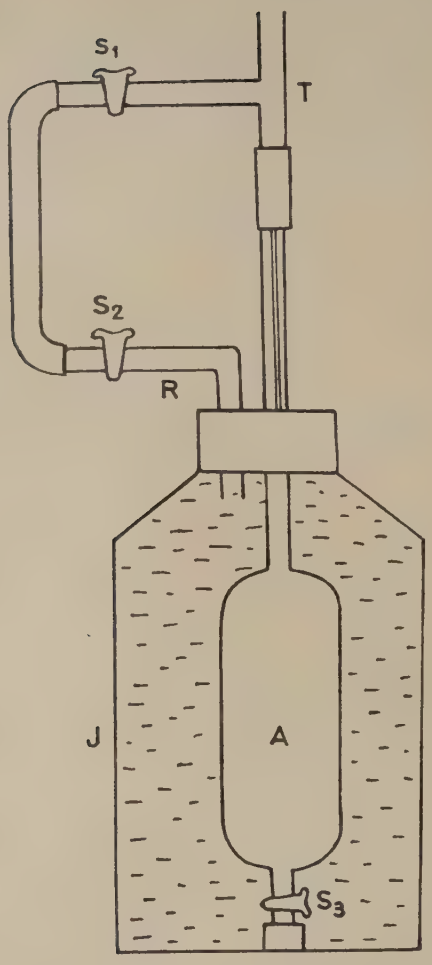

FIG. 1 — PIEZOMETER ASSEMBLY

stopcock S_1. To the bottom end of the bulb is connected a ground stopcock S_3 to facilitate quick filling and emptying. The bulb is enclosed in an outer metal jacket J, which is filled with water. The outer jacket is connected to the pressure system through the stopcocks S_2 and S_1. The dead space of the piezometer is limited to 4 or 5 cm. of the capillary tube (Fig. 1). The use of a fairly wide capillary tube minimizes the capillary effects.

The open end of the piezometer is connected to a pressure system and a low pressure of about 35 cm. Hg is produced using a vacuum pump. After allowing the liquid to settle at that pressure for some time, it is compressed adiabatically to the atmospheric pressure. The resulting shift of the liquid level in the capillary tube is measured using a microscope. The procedure is repeated a number of times and the average value of the shift is used to evaluate the change in the volume of the liquid.

Results

In Table 1, the values of compressibility of water obtained with the help of the piezometer are compared with the values obtained by other observers using static and dynamic methods. The variation of adiabatic compressibility with concentration for aqueous solutions of NaOH, KOH, $NaNO_3$, KNO_3 and $Na_2S_2O_3.5H_2O$ has been studied using the piezometric method (static) and the results are presented graphically in Fig. 2. The compressibility behaviour of these solutions has been studied previously by the

TABLE 1 — ADIABATIC COMPRESSIBILITY OF WATER AT 30°C.

Observer	Adiabatic compressibility $\times 10^6$ $atm.^{-1}$	
	Dynamic method	Static method
Parthasarathy	44·9	—
Tyrer	—	44·5
Philip	—	43·1
Author	44·6	43·0

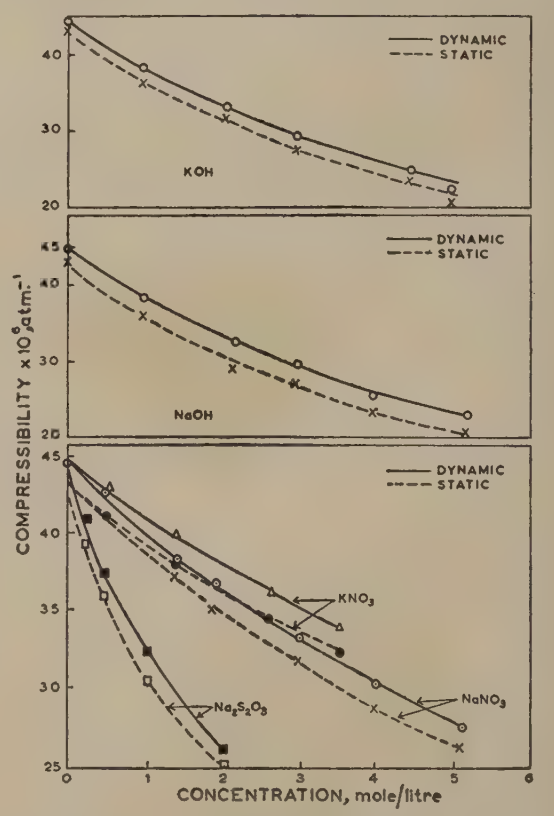

FIG. 2 — ADIABATIC COMPRESSIBILITY AS A FUNCTION OF CONCENTRATION FOR AQUEOUS SOLUTIONS OF DIFFERENT ELECTROLYTES

author[6-8] employing dynamic (ultrasonic) methods. These data are also presented in Fig. 2 for comparison. The absolute values of the compressibilities differ by about 3 per cent. In all the cases, the curves representing the static and dynamic behaviour of the solutions run almost parallel to each other. This indicates that the piezometer method gives a fairly accurate estimate of the rate at which the compressibility varies with concentration.

The results of measurements on aqueous solutions of lead nitrate and cadmium iodide are compared with the corresponding ultrasonic data in Fig. 3 and Table 2. Sound velocities at each concentration (col. 6, Table 2) are calculated from the compressibility data obtained by the static method (piezometer). They are given in column 6 of Table 2. The calculated velocities clearly show a decrease with increasing concentration, thus confirming the results obtained by the ultrasonic method (col. 5, Table 2). The agreement between the observed and calculated velocities is satisfactory.

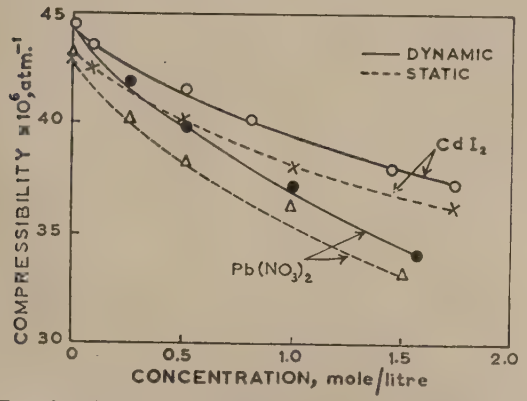

FIG. 3 — ADIABATIC COMPRESSIBILITY AS A FUNCTION OF CONCENTRATION FOR AQUEOUS SOLUTIONS OF LEAD NITRATE AND CADMIUM IODIDE

TABLE 2 — SOUND VELOCITIES FOR SOLUTIONS OF LEAD NITRATE AND CADMIUM IODIDE

Conc. *mole/litre*	Density *g./ml.*	Adiabatic compressibility ×10⁶ *atm.*⁻¹		Sound velocity *m./sec.*	
		Dynamic	Static	Obs.	Calc.
Lead nitrate					
0·00	0·996	44·50×10⁻⁶	43·0×10⁻⁶	1514	1538
0·25	1·073	41·93	40·2	1501	1532
0·50	1·140	39·97	38·2	1491	1525
1·00	1·271	36·95	36·5	1469	1478
1·54	1·405	33·89	33·0	1459	1479
Cadmium iodide					
0·13	1·036	43·50	42·4	1500	1518
0·50	1·147	41·67	40·3	1456	1480
1·00	1·285	39·25	38·1	1432	1457
1·75	1·476	37·10	36·2	1369	1377

Discussion

The velocity of sound in a fluid can be expressed as $V = (\beta\rho)^{-\frac{1}{2}}$, where ρ is the density of the medium and β is the adiabatic compressibility. A theoretical expression for the concentration dependence of sound velocity in electrolytic solutions can be arrived at by substituting in the above equation, the expressions for the concentration dependence of density[9] and compressibility[10]. Following such a procedure, we arrive at the equation $V = V_o + GC - HC^{3/2}$, where V is the velocity at molar concentration C, V_o the velocity in the solvent and G and H are constants. This equation closely fits the experimental results in a number of cases. At low concentrations the contribution of the term $HC^{3/2}$ is small, making the sound velocity a linear function of concentration. The constant G has positive values for all electrolytes, indicating an increase of sound velocity with increasing concentration. For aqueous solutions of lead nitrate and cadmium iodide the constant G is negative, indicating decreasing sound velocity with increase in concentration.

We can get an idea of the factors that contribute for this unusual behaviour by examining the nature of the constant G. This is related to the other thermodynamic properties of the solution by the equation

$$G = \frac{V_o}{2}\left[\frac{1}{\beta_o}\left(\frac{\delta\beta}{\delta c}\right)_\infty - \frac{1}{\rho_o}\left(\frac{\delta\rho}{\delta c}\right)_\infty\right]$$

where β_o is the compressibility of the pure solvent, ρ_o, the density of the solvent, $\left(\frac{\delta\beta}{\delta c}\right)_\infty$, the rate at which the compressibility varies with concentration at infinite dilution and $\left(\frac{\delta\rho}{\delta c}\right)_\infty$, the rate of variation of density at infinite dilution. The constants $\frac{1}{\beta_o}\left(\frac{\delta\beta}{\delta c}\right)_\infty$ and $\frac{1}{\rho_o}\left(\frac{\delta\rho}{\delta c}\right)_\infty$ have been evaluated for some electrolytic solutions and are given in Table 3. The factor representing the compressibility variation is larger than the corresponding factor for density variation in all the cases excepting lead nitrate and

TABLE 3 — RATE OF VARIATION OF ADIABATIC COMPRESSIBILITY AND DENSITY AT INFINITE DILUTION

Substance	$\frac{1}{\beta_o}\left(\frac{\delta\beta}{\delta c}\right)_\infty$	$\frac{1}{\rho_o}\left(\frac{\delta\rho}{\delta c}\right)_\infty$	G
KNO_3	0·093	0·070	+0·023
$NaNO_3$	0·120	0·071	+0·049
KOH	0·175	0·052	+0·123
NaOH	0·210	0·070	+0·140
$Pb(NO_3)_2$	0·298	0·318	−0·020
CdI_2	0·205	0·327	−0·122

cadmium iodide. In these two cases, the rate of variation of compressibility is the same as that for other electrolytes while the density changes rapidly. This leads to a negative value for the constant G. The heavy mass of the metallic ions is responsible for the rapid changes in density of these solutions and consequently for the decrease of sound velocity with increasing concentration. Further experimental studies with electrolytic solutions having heavy metallic ions are in progress.

Acknowledgement

The author takes this opportunity to offer his grateful thanks to Prof. S. Ramachandra Rao and Dr J. Bhimasenachar for their valuable suggestions and encouragement.

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Ultrasonic Relaxation due to Rotational Isomerism in Organic Liquids

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Ultrasonic absorption and velocities in some substituted ethanes, having rotational isomers with considerable energy difference, have been studied by a pulse method, in the frequency range 10-110 Mc/s., over the range of temperature from −70° to 100°C. The absorption data have been employed to obtain the relaxation parameters for these liquids, and to derive useful molecular information such as energy barriers involved in their internal rotation, energy difference between rotational isomers, etc.

ULTRASONIC relaxation due to rotational isomerism has been previously studied by Heasell and Lamb[1] in triethylamine and by De Groot and Lamb[2] in many aldehydes and ketones. The present investigation extends the study of ultrasonic relaxation to other known rotational isomers, such as ethane and its halogen substituted derivatives.

According to Davies and Lamb[3], whenever molecules of a liquid can reside in two or more equilibrium states which differ in energy (although they may have the same volume), it is possible for this equilibrium to be perturbed by the sound wave.

Consider, as an example, the case of a first order reaction

$$A_{12} \rightleftharpoons A_{21}$$

for which k_{12} and k_{21} are the appropriate rate constants. The important parameter as far as the sound wave is concerned is the relaxation time, τ, given by

$$\tau = (k_{12}+k_{21})^{-1}$$

At sufficiently low frequencies ($\omega\tau \ll 1$) the molecular distribution between the states A_1 and A_2 follows the variations of the sound wave. On the other hand, at very high frequencies ($\omega\tau \gg 1$) the internal equilibrium is sensibly not affected by the sound wave. As the frequency increases from a low

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value to higher values, when ω is of the order of $(1/\tau)$, there is an increasing phase retardation between the population density of a given energy state and the excess pressure variations of the sound wave. This is accompanied by a decrease in the magnitude of the changes in the population density with the result that the absorption per unit wavelength ($\mu = \alpha V/f$) (where α is the amplitude absorption coefficient) passes through a characteristic frequency f_c given by

$$f_c = \frac{k_{12}+k_{21}}{2\pi} \quad \ldots\ldots\ldots\ldots (1)$$

There is an accompanying decrease in the value of (α/f^2) from its low frequency value to a limiting high frequency value B when $f = f_c$. Such a behaviour is termed ultrasonic relaxation and with certain approximation can be represented by the equation

$$\frac{\alpha}{f^2} = \frac{A}{1+\frac{f^2}{f_c^2}} + B \quad \ldots\ldots\ldots\ldots (2)$$

where B represents the combined effects of absorption due to shear viscosity and any other relaxation process having a characteristic frequency much greater than f_c.

Taking the first term of equation (2) corresponding to ultrasonic absorption due to relaxation mechanism only and omitting the term B representing the 'classical' absorption due to viscosity, etc., μ, the absorption per wavelength due to relaxation is given by

$$\mu = \frac{AVf}{1+f^2/f_c^2} \quad \ldots\ldots\ldots\ldots (3)$$

and this attains a maximum value μ_m at $f = f_c$ and is given by

$$\mu_m = \tfrac{1}{2}AVf_c \quad \ldots\ldots\ldots\ldots (4)$$

If conditions are such that $\Delta G > 3RT$, that is, if the Gibbs free energy between the two assumed equilibrium states of the molecule is greater than $3RT$, then according to Heasell and Lamb[1] k_{12} can be neglected in comparison to k_{21} to within an accuracy of 5 per cent. Under these conditions,

$$f_c\left[=\frac{k_{21}}{2\pi}\right] = \frac{1}{2\pi}\left(\frac{KT}{h}\right)\left(e^{\frac{\Delta S_2}{R}}\right)\left(e^{-\frac{\Delta H_2}{RT}}\right) \quad \ldots\ldots (5)$$

where K is Boltzmann's constant, h is Planck's constant, ΔH_2, the energy of activation in the reverse direction, ΔS_2, the entropy of activation in the reverse direction, and R, the gas constant.

Thus ΔH_2 may be obtained from the linear plot of log (f_c/T) against $(1/T)$.

Substituting this value of ΔH_2 in equation (5) ΔS_2 may be obtained. Thus the reaction energy and entropy for the particular mechanism of relaxation assumed for the given molecule may be approximately estimated.

The energy difference between the two assumed equilibrium states can also be derived from the ultrasonic data if it be assumed that (vide Davies and Lamb) no change in volume occurs when the molecule passes from one state to another and that $\Delta G > 3RT$. In that case it has been shown by Heasell and Lamb[1]

$$\frac{2\mu_m}{\pi} = \frac{\bar{\gamma}-1}{\bar{C}_p} R\left(\frac{\Delta E}{RT}\right)^2 e^{-\frac{\Delta E}{RT}} e^{\frac{\Delta S}{R}} \quad \ldots\ldots (6)$$

where $R\left(\frac{\Delta E}{RT}\right)^2 e^{\frac{\Delta S}{R}} e^{-\frac{\Delta E}{RT}} = \Delta C_p$ is 'the relaxing specific heat', ΔE, the energy difference between the two assumed equilibrium states, ΔS, the entropy difference, $\bar{C}_p$, the specific heat, and

$$\bar{\gamma}-1 = \frac{V^2\beta^2 T}{JC_p} \quad \ldots\ldots\ldots\ldots (7)$$

where V is the velocity of the ultrasonic wave at temperature T°K., β, the expansion coefficient of the liquid at temperature T, and J, the mechanical equivalent of heat.

From this the value of μ_m is obtained as

$$\mu_m = \left[\frac{\pi}{2}\frac{\beta^2}{JR\bar{C}_p^2}(\Delta E)^2 e^{\frac{\Delta S}{R}}\right]\frac{V^2}{T}e^{-\frac{\Delta E}{RT}} \quad \ldots\ldots (8)$$

Making a further assumption (Padmanaban[4]) that the expansion coefficient and the specific heat of the liquid remain fairly constant over the temperature range under consideration,

$$\mu_m = \text{constant} \times \frac{V^2}{T} e^{-\frac{\Delta E}{RT}} \quad \ldots\ldots\ldots (9)$$

The plot of $\log\left(\frac{T\mu_m}{V^2}\right)$ against $1/T$ is thus linear and its slope gives $\Delta E/R$ and hence (ΔE), the energy difference between the two isomeric forms of the molecule.

Experimental procedure

The experimental arrangement used and the technique employed were the same as those described by Andreae[5], Heasell[6], Padmanaban[4] and Andreae *et al.*[7]. In principle, a short R/F pulse containing an appreciable number of oscillations at the frequency f is generated by a suitably modulated oscillator and is fed to a piezoelectric transducer. The resulting ultrasonic pulse passes through the liquid to be examined and is reconverted into an electrical pulse by a detecting transducer. After suitable amplification and demodulation the envelope of the received pulse is displayed on an oscilloscope. By varying the distance between the transmitting and detecting transducers and by measuring the change in amplitude of the received pulse, a plot of attenuation versus

path length is obtained. This gives the absorption coefficient α and hence the parameter α/f^2.

Results

A number of common organic liquids that were expected to show relaxation due to rotational isomerism were taken up for investigation. These were compounds originally suggested by Mizushima[8] as having more than one possible stable configuration revealed from spectroscopic, dielectric constant and electron diffraction studies. It is found that all the compounds having rotational isomerism taken up for investigation do not necessarily show ultrasonic relaxation within the range of frequency and temperature covered in this study.

Of about 30 liquids studied in the course of the investigation[4], six showed clear ultrasonic relaxation and were studied in detail. These were: (1) 1,2-dichloropropane (ClH_2C-$CHClCH_3$), (2) 1,2-dibromopropane (BrH_2C-$CHBrCH_3$), (3) 1,1,2-trichloroethane (Cl_2HC-ClH_2C), (4) 1,1,2-tribromoethane (Br_2HC-BrH_2C), (5) 1-bromopropane (BrH_2C-CH_2CH_3), and (6) 1,1,2,2-tetrachloroethane (sym) (Cl_2HC-$CHCl_2$).

Of these only the first four, viz. 1,2-dichloropropane, 1,2-dibromopropane, 1,1,2-trichloroethane and 1,1,2-tribromoethane, showed relaxation adequate for quantitative study.

The samples of the liquids 1,2-dichloropropane, 1,2-dibromopropane, 1,1,2-tribromoethane and 1-bromopropane were those supplied by Messrs Hopkin & William Ltd and the British Drug Houses Ltd, while 1,1,2-trichloroethane and 1,1,2,2-tetrachloroethane were supplied by the Imperial Chemical Industries Ltd on special request. Experiments were also conducted on samples of these liquids redistilled in the laboratory. The absorption results obtained thereby showed no significant difference from those obtained by measurements on the liquids supplied by the firms directly. Hence the compounds supplied by them were taken to be of sufficient purity for experimental purposes and the rest of the measurements were made on those samples obtained directly from the manufacturers.

The plots of α/f^2 against temperature at various frequencies for the six liquids* mentioned above studied were more or less similar in nature to that represented in Fig. 1 for 1,2-dichloropropane except those for 1,1,2,2-tetrachloroethane (liquid No. 6) which showed poor relaxation and gave curves similar to 1,1,2,2-tetrabromoethane (sym) studied by Krebs[9].

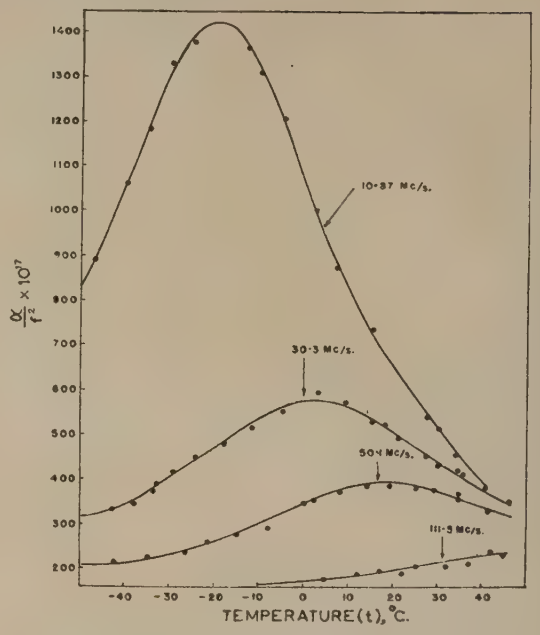

FIG. 1 — CURVES SHOWING ULTRASONIC RELAXATION IN 1,2-DICHLOROPROPANE

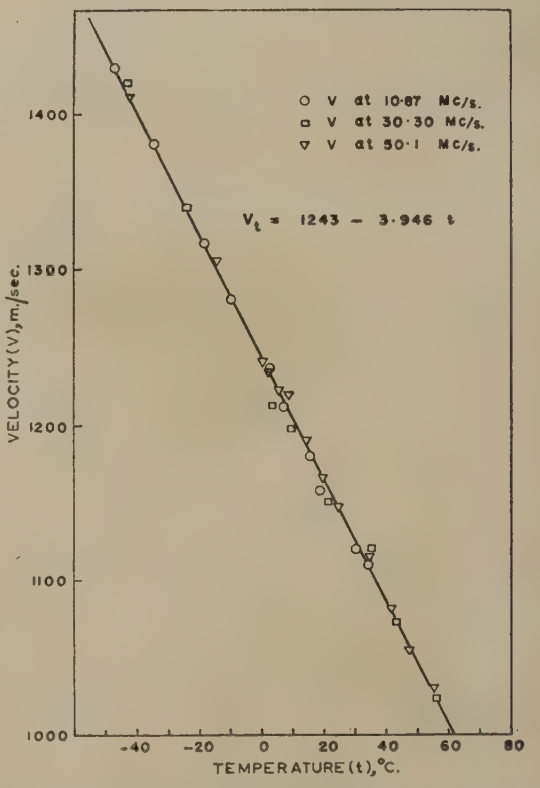

FIG. 2 — VARIATION OF VELOCITY WITH TEMPERATURE IN 1,2-DICHLOROPROPANE [○, velocity at 10·87 Mc/s.; □, velocity at 30·30 Mc/s.; and ▽, velocity at 50·10 Mc/s.]

*The graphs showing relaxation in these six liquids and the values of $\frac{\alpha}{f^2}$ and velocities at various temperatures can be had from the author.

From these data, the parameters f_c, A and B were estimated at regular intervals of temperature (10°C.) by the graphical method[4] explained below:

At a particular temperature choosing any three frequencies and the corresponding values of $\frac{\alpha}{f^2}$, the value of f_c was roughly estimated using the relation

$$\frac{\alpha}{f^2} = \frac{A}{1+\frac{f^2}{f_c^2}} + B$$

Then choosing a mid-frequency nearest to f_c thus obtained, and two other frequencies on either side, a better estimate of the value of f_c was made. These values of f_c were next plotted against temperature and the points joined by a smooth curve. From this graph the final values of f_c were read out, at the required temperatures, and used to calculate the parameters A and B. The values of A and B thus obtained were similarly plotted against temperature and the points joined by smooth curves. The final values of A and B were taken from this graph.

Velocity determinations were made at various temperatures in the above region at three different frequencies. The variation with temperature was found to be linear in each case as can be seen, for example, from the velocity-temperature graph for 1,2-dichloropropane (Fig. 2).

Knowing V, the velocity at any temperature, μ_m, the maximum absorption per wavelength could be estimated from the expression

$$\mu_m = \tfrac{1}{2} A V f_c$$

The values of f_c, A, B and μ_m for 1,2-dichloropropane, 1,2-dibromopropane, 1,1,2-trichloroethane, 1,1,2-tribromoethane and 1-bromopropane are given in Table 1.

The quantities in the last two columns, namely log (f_c/T) and log $(T\mu_m/V^2)$, are plotted against $1/T$, where T is the absolute temperature. These are shown in Figs. 3 and 4 respectively.

The slope of the log (f_c/T)-$(1/T)$ graph gives the value of ΔH_2, the activation energy in the reverse direction and the slope of the log $(T\mu_m/V^2)$ graph

TABLE 1 — ULTRASONIC VELOCITIES AND RELAXATION PARAMETERS IN THE LIQUIDS STUDIED

Liquid	Temp. °C.	Velocity m./sec.	f_c Mc/s.	$A\times10^{17}$ cm.$^{-1}$ sec.^{2}	$B\times10^{17}$ cm.$^{-1}$ sec.^{2}	$10^2\,\mu_m$	Log (f_c/T)	$10+\text{Log}\left(\frac{T\mu_m}{V^2}\right)$
1,2-Dichloropropane	−30	1361	10·0	2900	110	2·020	4·615	0·425
	−20	1322	13·0	2250	118	1·940	4·711	0·449
	−10	1283	17·5	1620	120	1·820	4·823	0·464
	0	1243	25·0	1110	120	1·730	4·961	0·486
	10	1203	36·0	760	113	1·650	5·104	0·508
	20	1164	50·5	560	104	1·650	5·235	0·553
	30	1125	70·5	420	96	1·665	5·367	0·600
	40	1085	98·0	320	82	1·700	5·495	0·655
	50	1045	143·0	250	75	1·870	5·646	0·743
1,2-Dibromopropane	−20	1110	8·5	6600	230	3·110	4·526	0·806
	−10	1082	12·5	4420	205	2·920	4·649	0·817
	0	1054	18·0	2940	180	2·890	4·819	0·851
	10	1026	25·5	2100	160	2·770	4·954	0·874
	20	998	35·0	1540	145	2·690	5·077	0·899
	30	970	47·0	1140	135	2·600	5·190	0·923
	40	942	62·0	860	125	2·510	5·297	0·947
	50	915	83·0	670	118	2·540	5·410	0·991
1,1,2-Trichloroethane	0	1244	5·5	6000	60	2·050	4·303	0·559
	10	1210	9·5	3800	60	2·170	4·530	0·623
	20	1176	14·0	2770	60	2·280	4·680	0·684
	30	1142	19·5	2060	70	2·290	4·810	0·727
	40	1108	27·0	1590	80	2·380	4·940	0·785
	50	1074	37·0	1220	90	2·420	5·060	0·831
	60	1040	48·5	940	115	2·370	5·160	0·863
	70	1006	64·5	720	150	2·340	5·270	0·899
	80	972	87·5	520	190	2·220	5·390	0·919
	90	938	126·0	400	240	2·360	5·540	0·988
1,1,2-Tribromoethane	20	1030	6·3	6650	156	2·160	4·332	0·776
	30	1006	9·5	4400	153	2·100	4·497	0·799
	40	981	13·5	3100	158	2·050	4·634	0·825
	50	956	18·7	2250	150	2·010	4·763	0·851
	60	932	25·8	1550	175	1·980	4·889	0·883
	70	907	36·0	1240	170	2·030	5·021	0·927
	80	882	50·0	950	170	2·090	5·152	0·978
1-Bromopropane	−50	1200	12·5	830	67	0·623	4·740	$\bar{1}$·984
	−40	1168	22·0	550	60	0·705	4·975	$\bar{1}$·079
	−30	1136	34·0	350	54	0·675	5·146	$\bar{1}$·104
	−20	1104	46·5	200	57	0·515	5·265	$\bar{1}$·029
	−10	1072	61·0	90	65	0·290	5·365	$\bar{1}$·826

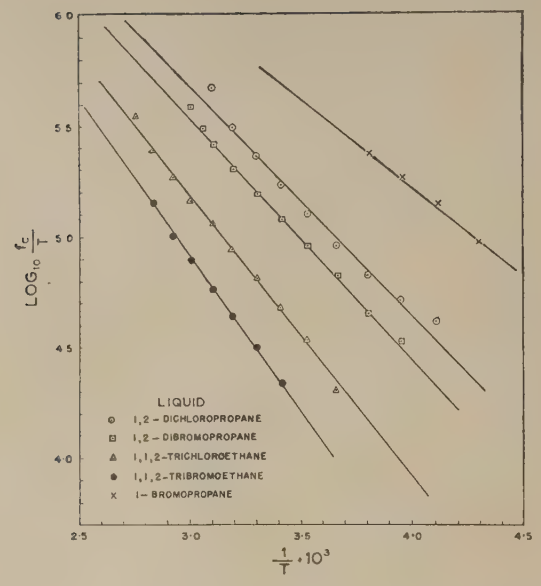

FIG. 3 — PLOT OF LOG (f_c/T) VERSUS $1/T$ FOR DIFFERENT LIQUIDS [⊙, 1,2-dichloropropane ($\Delta H_2 = 4{\cdot}75$ kcal./mole); ⊡, 1,2-dibromopropane ($\Delta H_2 = 4{\cdot}9$ kcal./mole); △, 1,1,2-trichloroethane ($\Delta H_2 = 5{\cdot}8$ kcal./mole); ●, 1,1,2-tribromoethane ($\Delta H_2 = 6{\cdot}4$ kcal./mole); and ×, 1-bromopropane ($\Delta H_2 = 3{\cdot}6$ kcal./mole)]

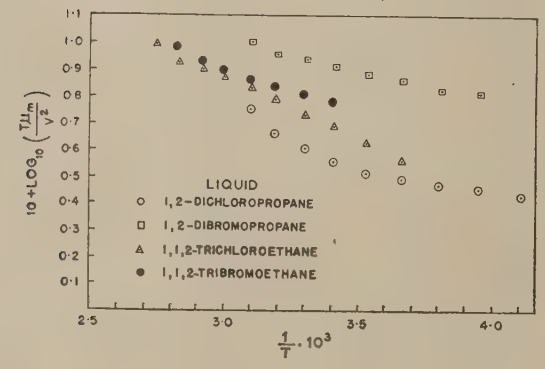

FIG. 4 — PLOT OF LOG $(T\mu_m/V^2)$ VERSUS $1/T$ FOR DIFFERENT LIQUIDS [⊙, 1,2-dichloropropane (av. $\Delta E = 1{\cdot}1$ kcal./mole); ⊡, 1,2-dibromopropane (av. $\Delta E = 0{\cdot}87$ kcal./mole); △, 1,1,2-trichloroethane (av. $\Delta E = 2{\cdot}15$ kcal./mole); and ●, 1,1,2-tribromoethane (av. $\Delta E = 1{\cdot}6$ kcal./mole)]

TABLE 2 — CHARACTERISTIC MOLECULAR PARAMETERS, ΔH_2, ΔS_2 AND ΔE

ORGANIC LIQUID	ENERGY OF ACTIVATION IN THE REVERSE DIRECTION (ΔH_2) *kcal./mole*	ENTROPY OF ACTIVATION IN THE REVERSE DIRECTION (ΔS_2) *cal./mole/°C.*	ENERGY DIFFERENCE BETWEEN TWO ISOMERS (ΔE) *kcal./mole*
1,2-Dichloropropane	4·7	−3·7	0·7 to 2·4
1,2-Dibromopropane	4·9	−3·7	0·9
1,1,2-Trichloroethane	5·8	−2·6	2·1
1,1,2-Tribromoethane	6·4	−2·1	1·6
1-Bromopropane	3·6	−13·6	—

gives the energy difference between the rotational isomers of the respective molecules.

The results are summarized in Table 2.

Acknowledgement

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Spectrographic Estimation of Hafnium & Zirconium in Their Binary Oxide Mixtures

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A procedure is described for the quantitative spectrographic estimation of Hf/Zr and Zr/Hf ratios in binary oxide mixtures using the intermittent a.c. arc excitation technique. Five selected line pairs cover the range 0·03-64 per cent Hf/Zr and 0·1-90 per cent Zr/Hf (wt/wt). The method has been applied to the analysis of hafnia in a sample of Travancore zircon and of the minor constituent in a large number of binary oxide fractions obtained in a scheme of chromatographic separation of zirconium from hafnium.

THE study of any fractionating process for the separation of pure zirconium and pure hafnium from a mixture of the two involves analysis of either constituent over a wide range of concentration. Satisfactory chemical methods are not available and recourse is taken to spectrographic or radiotracer methods. For obvious reasons, most of the published papers on the spectrochemical analysis of these mixtures relate to the determination of small amounts of hafnium in zirconium. The determination of small amounts of zirconium in hafnium becomes important only when a process for making pure hafnium is under development[1].

Feldman[2] reported a method for the determination of Hf/Zr weight ratio over the range 0·07-9·3 per cent based on the porous cup technique. Fassel and Anderson[3] employed the 'conducting briquette' technique over a wider range with the help of several line pairs. Kingsbury and Temple[4] used the intermittent a.c. arc technique for determining HfO_2 contents in a binary oxide mixture over the range 0·01-55 per cent with two line pairs. A comprehensive study on the determination of both ZrO_2/HfO_2 and HfO_2/ZrO_2 ratios by d.c. arc and 8000 V. spark excitations was reported by Gusyatskaya and Russanov[5]. For extremely low concentration of hafnia (>0·003 per cent), Mortimore and Noble[6] reported a d.c. arc method in which the graphite arc is stabilized by adding to the sample a 1 : 1 mixture of powdered graphite and barium fluoride.

In the present study, the intermittent a.c. arc technique[7] has been adopted since it combines the advantage of good reproducibility with high sensitivity; further, a small quantity of sample is required (5 mg.). Optimum conditions under which this technique can be used for the determination of Hf/Zr and Zr/Hf weight ratios in the ranges 0·03-64 and 0·1-90 per cent respectively are reported in this paper. Indian zircon (from Travancore) has been analysed (for Hf content) under these conditions in addition to a large number of oxide mixtures of known and unknown compositions.

Experimental procedure

Preparation of standards — Spectroscopically pure hafnium and zirconium oxides were dissolved by fuming with a mixture of sulphuric acid and a little hydrofluoric acid in a platinum crucible. After dilution, the hydroxides were precipitated from solution by gaseous ammonia, washed and centrifuged, and dissolved in hydrochloric acid to make up to known volumes in standard volumetric flasks. The hafnium and zirconium contents of these solutions were checked again by carrying out gravimetric estimations from aliquots. The standard solutions were then mixed in required proportions to give appropriate hafnium standards (Hf/Zr 0·032-64 per cent) and zirconium standards (Zr/Hf 0·1-90 per cent), the hydroxides precipitated with a little ashless paper pulp, filtered, and ignited to oxide in a muffle at 750-800°C. The ignited oxides were agated before taking on electrodes for excitation.

Preparation of samples for analysis — The final samples for analysis were always in the oxide form. A 5 mg. sample was placed in the cavity of the lower electrode, and a drop or two of a solution of phenol-

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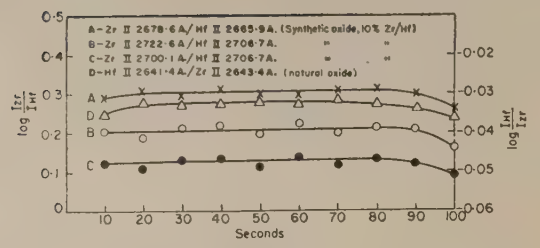

FIG. 1 — TIME-STUDY CURVES FOR NATURAL AND SYNTHETIC OXIDES USING DIFFERENT LINE PAIRS

TABLE 1 — SELECTED LINE PAIRS

Special line pairs *A.*	Intensities (M.I.T. tables) Arc	Spark	Range found applicable *wt* %	Remarks
Hf II 2641·406 Zr II 2643·395	40 5	125 5	0·032-64 Hf/Zr —	Good reproducibility
Hf II 2606·372 Zr II 2643·395	25 5	50 5	1·065-64 Hf/Zr —	do
Zr II 2678·632 Hf II 2665·966	80 20	100 35	0·1-90 Zr/Hf —	Good reproducibility. No interference from Hf II 2678·426 or Zr II 2665·177
Zr II 2722·610 Hf II 2706·727	50 10	50 50	0·1-90 Zr/Hf —	Good reproducibility. No interference from Zr I 2706·367
Zr II 2700·131 Hf II 2706·727	50 10	50 50	0·1-90 Zr/Hf —	Good reproducibility. No interference

formaldehyde resin in acetone added and dried in air to fix the oxide powder. The electrodes thus loaded were finally baked in an air-oven at 110°C. for an hour before being used for excitation.

Using the intermittent a.c. arc excitation, 5, 10 and 20 mg. oxide samples were exposed separately each for 45 sec. The 5 mg. sample was found sufficient for producing desired blackening of the selected line pairs on photographic plates.

Electrodes — Long carbon electrodes of spectroscopic quality (special RW III from Messrs Ringsdorffwerke, West Germany) were cut to 50 mm. (×7 mm. diam.) pieces and used. The lower electrode had a $\frac{3}{16}$ in. deep cavity of $\frac{1}{16}$ in. diam. while the upper electrode had a flat surface. There was no special advantage in having the upper electrode pencil-shaped.

Choice of line pairs — For oxide mixtures over the entire range, a number of line pairs were scrutinized by means of moving plate and reproducibility studies. In the moving plate studies, 25 mg. samples were arced for 100 sec. and 10 successive exposures were taken by racking the plate holder every 10 sec. From the 10 spectra thus obtained, time-study curves were drawn for selected line pairs (Fig. 1). Reproducibility tests were then carried out with them at the optimum exposure time, viz. 45 sec. (after pre-arcing for 10 sec.), to check constancy of the density ratio as well as the log intensity ratio over 8 exposures. The line pairs finally selected are given in Table 1.

Excitation of specimens — Littrow type large quartz spectrograph (Hilger E 492), with Hilger F 1025 lens, 2 cm. from the slit, was used. An intermittent 4-4·5 amp. a.c. arc was used for excitation and a high frequency spark from a Tesla coil was used for initiating the arc.

The following excitation conditions were employed: distance of source from slit, 38 cm.; slit width, 0·015 mm.; slit height, 2 mm.; electrode separation, 4 mm. (adjusted by using Hilger gauge lens F 1167); wavelength setting, 2400-3350 A.; and pre-arcing period, 10 sec.

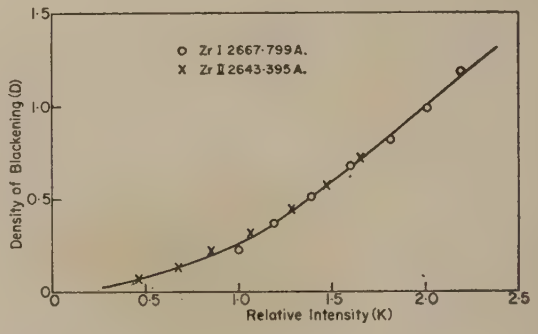

FIG. 2 — PLATE CALIBRATION CURVE (ILFORD ORDINARY PLATE)

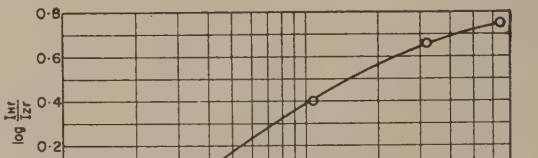

FIG. 3 — ANALYTICAL WORKING CURVE: $\frac{\text{Hf}}{\text{Zr}} = 1{\cdot}06\text{-}64$ PER CENT (WT/WT); $\frac{\text{Hf II } 2641{\cdot}4 \text{ A.}}{\text{Zr II } 2643{\cdot}4 \text{ A.}}$ (ILFORD ORDINARY PLATE)

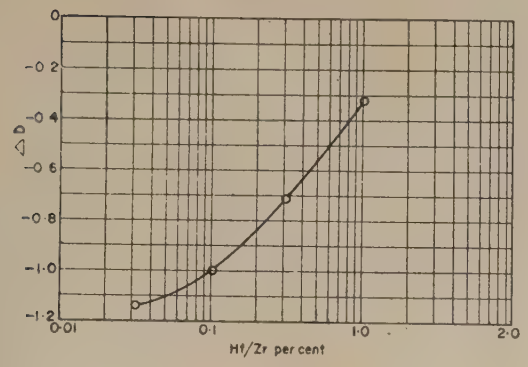

FIG. 4 — ANALYTICAL WORKING CURVES: $\frac{Hf}{Zr}$ = 0·032-1·06 PER CENT (WT/WT); $\frac{Hf\ II\ 2641{\cdot}4\ A.}{Zr\ II\ 2643{\cdot}4\ A.}$ ((ILFORD ZENITH PLATE)

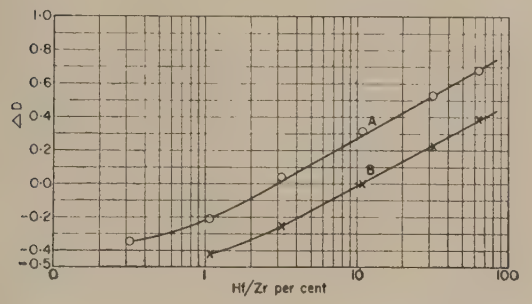

FIG. 5 — ANALYTICAL WORKING CURVES: (A) $\frac{Hf}{Zr}$ = 0·32-64 PER CENT (WT/WT); $\frac{Hf\ II\ 2641{\cdot}4\ A.}{Zr\ II\ 2643{\cdot}4\ A.}$; (B) $\frac{Hf}{Zr}$ = 1·06-64 PER CENT (WT/WT); $\frac{Hf\ II\ 2606{\cdot}4\ A.}{Zr\ II\ 2643{\cdot}4\ A.}$ (ILFORD ORDINARY PLATE)

Exposure period — By using a seven-step sector (exposure ratio 1 : 2), the exposure period was fixed at 45 sec. With this exposure, the density range of the selected line pairs was found convenient, and the transmission for background in the neighbourhood of the lines ranged between 86 and 96 per cent. Increasing the exposure to 90 sec. had no significant advantage either with low hafnia or low zirconia samples.

Development of plates — Ilford ordinary plates were used for Hf/Zr range 0·32-64 per cent (wt/wt) and Zr/Hf range 0·1-90 per cent (wt/wt) and Ilford Zenith plates for Hf/Zr range 0·032-10 per cent (wt/wt). The exposed plates were developed for 2 min. at 18-19°C. using ID2 developer (1 : 2). The fixing period was 15 min.

Densitometry — Hilger non-recording microphotometer, set at clear plate deflection of 50, was used.

Analytical working curves — Plate calibration was done with a seven-step filter following the method of

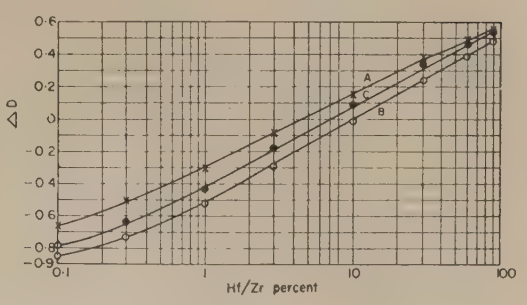

FIG. 6 — ANALYTICAL WORKING CURVES: $\frac{Hf}{Zr}$ = 0·1-90 PER CENT (WT/WT); (A) $\frac{Zr\ II\ 2678{\cdot}6\ A.}{Hf\ II\ 2665{\cdot}9\ A.}$; (B) $\frac{Zr\ II\ 2700{\cdot}1\ A.}{Hf\ II\ 2706{\cdot}7\ A.}$; (C) $\frac{Zr\ II\ 2722{\cdot}6\ A.}{Hf\ II\ 2706{\cdot}7\ A.}$ (ILFORD ORDINARY PLATE)

TABLE 2 — INTERFERENCE DUE TO Fe AND Ti

(*Line pair Hf* 2641·406-*Zr* 2643·395)

Fe IN MATRIX %	LOG (I_{Hf}/I_{Zr})	Ti IN MATRIX %	LOG (I_{Hf}/I_{Zr})
1·04	+0·026	1·01	+0·027
3·12	+0·028	3·03	+0·026
10·40	+0·025	10·10	+0·028
31·20	−0·075	30·30	+0·029
62·40	−0·060	60·60	+0·023

Nickelson and Stubbs[8]. Fig. 2 shows a plate calibration curve obtained by using Ilford ordinary plate. An analytical working curve was drawn (Fig. 3) by plotting log intensity ratios of the selected line pair against corresponding weight percentages of the metal in the synthetic standards, after correcting for the relative intensity of the background derived from the plate calibration curve. Subsequently, differences in the density values (after deduction of background densities) of the selected line pair were directly plotted against the corresponding weight percentages of the metal on a semi-log paper. Figs. 4-6 show the analytical working curves drawn in this manner.

Estimation of hafnium in Travancore zircon—Zircon mineral (0·5 g.) was agated and decomposed by fusion for 0·5 hr in a nickel crucible with sodium peroxide reagent (5 g.). The fused mass was cooled and treated with 1 : 1 hydrochloric acid (50 ml.), evaporated to dryness and baked on a sand bath to dehydrate silica. The residue was taken up in dilute hydrochloric acid and filtered. Zirconium-hafnium hydroxide was then precipitated from the filtrate with excess of ammonia gas, washed with hot 2 per cent ammonium nitrate, and ignited at 750-800°C. to oxide. The ignited oxide was agated, 5 mg. quantities taken on the

electrodes and arced as already described. The line pair Hf II 2641·4/Zr II 2643·4 was used for density measurements. The results of analysis of hafnium in the 'natural' oxide [$Zr(Hf)O_2$= 66·8 per cent] from Indian zircon showed the Hf/Zr ratio (weight per cent) in natural oxide to be 3·0 ± 0·15 and the weight of HfO_2 in the mineral to be 1·7 per cent (mean of six independent determinations). Since the possible metallic contaminants in the mixed oxide are iron and titanium, the interference due to these two elements was studied. The results are presented in Table 2.

About 200 samples of mixed oxides $Zr(Hf)O_2$ representing fractions (Hf/Zr 0·1-62 and Zr/Hf 0·1-20 per cent), obtained in our work on the separation of zirconium from hafnium[1], were also analysed under the optimum conditions arrived at in this paper.

Accuracy of results — A statistical analysis of the results with synthetic samples containing zirconia-hafnia in the range 0·032-64 per cent Hf/Zr and 0·1-90 per cent Zr/Hf showed that analyses could be done over these two ranges within a standard deviation of 5-8·8 per cent (Table 3). Beyond these limits, the linearity of the relationships shown in the curves (Figs. 3-6) does not hold good under the established conditions. For most of the analyses, Ilford ordinary plates were found quite suitable. For low hafnia samples (Hf/Zr 0·032-1·0 per cent), more consistent density estimates could be made on Ilford Zenith plates.

TABLE 3 — SPECTROCHEMICAL ANALYSIS OF HAFNIA-ZIRCONIA MIXTURE

(Ilford ordinary photographic plates were used in all cases except in the case of first mixture where Ilford Zenith plates were used)

Conc. range *wt* %	Line pairs used *A.*	Standard deviation* %
Hf/Zr 0·032-1·06	Hf II 2641·4 / Zr II 2643·4	8·8
Hf/Zr 1·06-30	Hf II 2641·4 / Zr II 2643·4	5·0
	Hf II 2606·3 / Zr II 2643·4	6·2
Hf/Zr 30	Hf II 2641·4 / Zr II 2643·4	7·2
	Hf II 2606·3 / Zr II 2643·4	7·0
Zr/Hf 0·1-10	Zr II 2678·7 / Hf II 2665·9	5·0
	Zr II 2700·1 / Hf II 2706·7	5·2
	Zr II 2722·6 / Hf II 2706·7	5·0
Zr/Hf 10-90	Zr II 2678·7 / Hf II 2665·9	6·0
	Zr II 2700·1 / Hf II 2706·7	6·2
	Zr II 2722·6 / Hf II 2706·7	6·0

*Calculated on six independent duplicates. For Hf/Zr = 3 per cent, the deviation, calculated over 30 duplicates, remained unchanged.

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Partial Methyl Ethers of Polyhydroxy Coumarins: Synthesis of Isofraxidin & Fraxetin & Ring Isomeric Change in Coumarins

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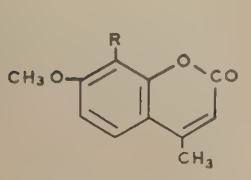
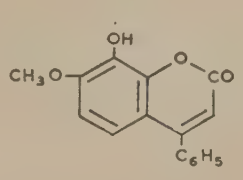
In continuation of earlier work on the synthesis of fraxidin and adopting Dakin's oxidation of 8-acetyl coumarins, 4-methyl-7-methoxy-8-hydroxy coumarin and 4-phenyl-7-methoxy-8-hydroxy coumarin have been prepared. Improved synthesis of isofraxidin and fraxetin could be effected by this method. In the course of this work partial demethylation and ring isomeric change have been observed in the oxidation of 4-methyl-5,7-dimethoxy-8-acetyl coumarin and the product has been shown conclusively to be 4-methyl-5,6-dihydroxy-7-methoxy coumarin. In the case of 4-methyl-5-methoxy-7-hydroxy-8-acetyl coumarin, two conditions have been adopted : in the cold with restricted amount of alkali, there is simple Dakin's oxidation yielding 4-methyl-5-methoxy-7,8-dihydroxy coumarin ; but if excess of alkali is used and initial heating carried out as in the case of non-hydroxy coumarins, the same demethylation and isomeric change takes place to yield 4-methyl-5,6,7-trihydroxy coumarin. Experiments indicate that alkali treatment alone does not produce the demethylation and the influence of hydrogen peroxide seems to be involved. Ring isomeric change takes place readily in alkaline medium as is shown by the conversion of 4-methyl-5,7,8-trihydroxy coumarin into the corresponding 5,6,7-trihydroxy isomer in nitrogen atmosphere.

IN a recent publication[1] it was shown that Dakin's reaction can be conveniently applied to an acetyl coumarin having no free hydroxyl group. By this method 7-methoxy-8-acetyl coumarin gave a good yield of 7-methoxy-8-hydroxy coumarin. Under the alkaline conditions employed, the coumarin ring opens and the hydroxyl group thus liberated helps in the oxidation. This method was also applied for a convenient synthesis of fraxidin. Its utility has now been examined for the preparation of 4-methyl- and 4-phenyl-substituted daphnetin derivatives and related compounds. 4-Methyl-7-methoxy-8-acetyl coumarin (I) yielded 4-methyl-daphnetin-7-methyl ether (II) in good yield. This compound could also be prepared by the partial demethylation of the dimethyl ether. Similar series of reactions could be carried out with a phenyl group in the 4-position yielding 4-phenyl-daphnetin-7-methyl ether (III).

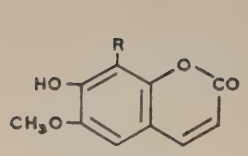


I, R = CO CH3
II, R = OH

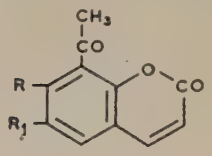


III

Attempts have next been made to synthesize isofraxidin (IV) and fraxetin (V). 8-Acetyl-umbelliferone (VI) was the convenient starting material. Its 7-benzyl ether (VII) when subjected to persulphate oxidation gave rise to 6-hydroxy-7-benzyloxy-8-acetyl coumarin (VIII). In this reaction it was noticed that partial debenzylation also took place giving rise to 6,7-dihydroxy-8-acetyl coumarin which on methylation gave the known dimethyl ether[1]. It is difficult to say when exactly debenzylation takes place; the most probable stage is the hydrolysis of the sulphate with hydrochloric acid. It is also possible that it took place earlier in the alkaline solution under the influence of the oxidizing agent; a similar reaction is reported elsewhere in this paper. The predominant product, however, was 6-hydroxy-7-benzyloxy-8-acetyl coumarin (VIII) which was



IV, R = OCH_3
V, R = OH



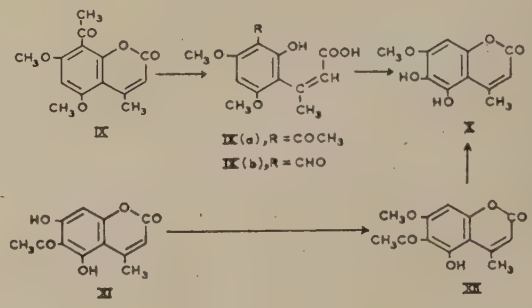
VI, R = OH; R_1 = H
VII, R = OC_7H_7; R_1 = H
VIII, R = OC_7H_7; R_1 = OH

methylated. When subsequently Dakin's reaction and debenzylation (hydrogenolysis) were carried out, fraxetin (V) was obtained. On the other hand, if methylation of the 8-hydroxy group was done before final debenzylation, isofraxidin (IV) was produced.

Isofraxidin was isolated along with its isomer fraxidin by Späth and co-workers[2] from the bark of German ash wood. A mixture of these two was also obtained[3] by partial methylation of fraxetin and the separation of the mixture was carried out with difficulty. The present synthesis of isofraxidin by independent synthesis makes it more readily available. Fraxetin, which is also found in Fraxinus species[4], was originally synthesized by Späth *et al.*[5,6] in poor yields. Later synthesis by Aghoramurthy and Seshadri[7] was a considerable improvement and the present method cuts down the number of steps further, making the procedure convenient.

In an earlier paper Sastri *et al.*[8] showed that demethylation of 5,7,8-trimethoxy-4-methyl coumarin and 5,6,7-trimethoxy-4-methyl coumarin with boiling hydriodic acid under ordinary conditions did not bring about any isomeric change; only the corresponding hydroxy compounds were obtained. This was explained as due to the non-opening of the α-pyrone ring in the boiling acid. Since this ring opens out more readily in alkaline medium, we have now come across this change in the course of the present work. In the somewhat analogous case of isoflavones partial methyl ethers have been successfully used[9] and isomeric change in alkaline medium has been found to be useful even for preparative purposes[10].

During the study of alkaline Dakin's oxidation of acetyl coumarins (the oxidation was unsuccessful under acid conditions) it was noticed that 4-methyl-5,7-dimethoxy-8-acetyl coumarin (IX) unexpectedly gave rise to a dihydroxy coumarin derivative. It contained only one methoxyl and on methylation gave 4-methyl-5,6,7-trimethoxy coumarin. Its constitution as 4-methyl-5,6-dihydroxy-7-methoxy coumarin (X) was confirmed by comparison with an authentic sample prepared by the following procedure: 4-Methyl-5,7-dihydroxy-6-acetyl coumarin (XI) was subjected to monomethylation. The product was found to be the same as the one obtained by the condensation of phloracetophenone-2-methyl ether with ethyl acetoacetate. Based on analogy[8] with the coumarins obtained from resacetophenone, the synthetic product could be given the structure 4-methyl-5-hydroxy-6-acetyl-7-methoxy coumarin (XII). Hence the monomethylation has taken place in position 7. This result of partial methylation could be explained as due to the preferred chelation between the 5-hydroxyl and 6-acetyl groups of the coumarin leading to the protection of this hydroxyl group. The above structure is supported by the application of the Dakin's reaction to the monomethyl ether yielding 4-methyl-5,6-dihydroxy-7-methoxy coumarin (X); this agreed with a sample of the compound prepared by an earlier method of Sawhney and Seshadri[11] using a different route starting from 4-methyl-6-hydroxy-7-methoxy coumarin.



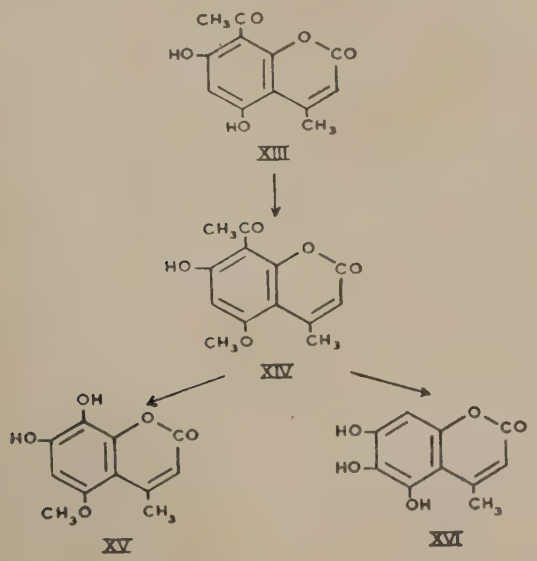
IX
IX(a), R = $COCH_3$
IX(b), R = CHO
X
XI
XII

It was, therefore, evident that during Dakin's oxidation of (IX), which in the strong alkaline solution had been converted into coumarinic acid (IXa), there is simultaneous demethylation, and when the final ring closure takes place it leads to the isomeric product. However, coumarin (IX), on similar treatment only with hot alkali and without adding hydrogen peroxide, did not undergo any change. Hence the action of alkali alone does not bring about the demethylation and hydrogen peroxide is also necessary.

The above-mentioned 4-methyl-5,6-dihydroxy-7-methoxy coumarin (X) dissolves in aqueous alkali and is recovered unchanged after acidification. Hence there is no reverse isomeric change and 5,6,7 arrangement would represent a stable structure. It may be mentioned here that Naik and Thakor[12] reported that 4-methyl-5,7-dimethoxy-8-hydroxy coumarin (m.p. 258°) is obtained by Dakin's reaction of 3-formyl-4,6-dimethoxy-β-methyl coumarinic acid (IXb), but they did not establish the constitution of their product. Based on the results obtained above, it could be concluded that their product should be coumarin (X). The melting point given by

them agrees with that of 4-methyl-5,6-dihydroxy-7-methoxy coumarin.

The above observation of the isomeric change was also corroborated by the following experiment. When 4-methyl-5,7-dihydroxy-8-acetyl coumarin (XIII) was methylated with one mole of dimethyl sulphate and potassium carbonate, the monomethyl ether could not be obtained. The acetone solution yielded only the dimethyl ether, whereas from the solid carbonate after treatment with hydrochloric acid could be obtained only unchanged material. This result may be explained as due to the ready formation of the dipotassium salt of the ketone (XIII) which directly gets converted into the dimethyl ether. The monomethyl ether could, however, be easily prepared by using sodium bicarbonate instead. The resulting compound was 4-methyl-5-methoxy-7-hydroxy-8-acetyl coumarin (XIV) because it gave a positive ferric reaction indicating the presence of a chelated hydroxyl group which had escaped methylation. This conclusion was supported by the application of the Dakin's oxidation (in the cold) forming an *o*-dihydroxy compound, giving a strong ferric reaction and capable of forming a methylenedioxy derivative. The product should, therefore, be 4-methyl-5-methoxy-7,8-dihydroxy coumarin (XV). However, when the acetyl coumarin (XIV) was subjected to Dakin's oxidation (in the hot) as in the case of 4-methyl-5,7-dimethoxy-8-acetyl coumarin (IX) it gave rise to 4-methyl-5,6,7-trihydroxy coumarin (XVI), again showing that ring isomeric change had taken place as the result of demethylation. There was no change on heating with sodium hydroxide solution alone.



The isomerization of 4-methyl-5,7,8-trihydroxy to 5,6,7-trihydroxy coumarin has also been studied. The former on treatment with aqueous alkali (100°; 30 min.) in nitrogen atmosphere underwent isomeric change to give 4-methyl-5,6,7-trihydroxy coumarin. The identity of the trihydroxy coumarin in this and the earlier experiment was established by mixed melting point and comparison of the ultraviolet absorption spectra with those of authentic samples of 5,6,7- and 5,7,8-trihydroxy-4-methyl coumarins. The ultraviolet spectra of their derivatives have also been recorded and used in the characterization.

In an attempt to study the isomerization of 4-methyl-5,7-dihydroxy-8-acetyl coumarin (XIII), it was treated with aqueous sodium hydroxide in the cold but there was no change. But on heating for 1½ hr at 100°, the product was found to be 4-methyl-5,7-dihydroxy coumarin, obviously arising by simple deacylation. As already mentioned, if one of the two hydroxyl groups is methylated no such deacylation takes place.

Recently Wheeler and co-workers[13] have reported that 5,7,8-trimethoxy and trihydroxy coumarins could be made to undergo change into 5,6,7-trihydroxy compounds when heated with strong hydriodic acid at elevated temperatures and high pressures. The present alkali method is, however, more smooth and gives good yield.

Dakin's oxidation in acid medium has been successfully carried out by Schönberg[14] using methoxy coumarin aldehydes. As another example, this procedure has been used in the present work for the conversion of 4-methyl-5-formyl-6-hydroxy-7-methoxy coumarin into 4-methyl-5,6-dihydroxy-7-methoxy coumarin. The method, however, does not work with the ketones mentioned in this paper.

Experimental procedure

4-Methyl-7-methoxy-8-hydroxy coumarin (II) — (a) *Dakin's oxidation* — 4-Methyl-7-methoxy-8-acetyl coumarin[15] (1 g.) was heated on a boiling water bath with aqueous sodium hydroxide (10 ml.; 4 per cent) for 15 min. The clear solution was cooled to 5° and hydrogen peroxide (1 ml.; 30 per cent) added during the course of 15 min. The mixture was left for 2 hr and acidified with dilute hydrochloric acid. The solid product (0·65 g.) crystallized from methanol as colourless rectangular plates, m.p. 160-1°. Naik and Thakor[12] reported m.p. 156°. Mixed melting point with the product obtained in (b) was undepressed.

(b) *Partial demethylation* — 4-Methyl-7,8-dimethoxy coumarin[16] (0·5 g.) was heated (110°) with hydriodic acid (10 ml.) in acetic anhydride (10 ml.) for 30 min. The mixture was poured into cold

saturated sodium bisulphite solution (50 ml.) and the product that separated (0·3 g.) crystallized from methanol, m.p. 160-1°.

On methylation with dimethyl sulphate and potassium carbonate in anhydrous acetone solution it gave 4-methyl-7,8-dimethoxy coumarin, m.p. and mixed m.p. 132-3°.

4-Phenyl-7-methoxy-8-hydroxy coumarin (III)— 4-Phenyl-7-methoxy-8-acetyl coumarin[17] (1 g.) was dissolved in hot aqueous sodium hydroxide (10 ml., 4 per cent), the solution cooled (5°) and treated slowly with hydrogen peroxide (1 ml., 30 per cent). After 2 hr, it was acidified with dilute hydrochloric acid; the solid product (0·6 g.) crystallized from methanol as colourless rectangular plates, m.p. 170-1°. (Found: C, 71·6; H, 5·2. $C_{16}H_{12}O_4$ requires C, 71·6; H, 4·5%.) Mixed melting point with a sample obtained by partial demethylation of 4-phenyl-7,8-dimethoxy coumarin[18] with hydriodic acid in acetic anhydride as given above was undepressed. On methylation with dimethyl sulphate and potassium carbonate method it gave 4-phenyl-7,8-dimethoxy coumarin, m.p. and mixed m.p. 136-7°.

7-Benzyloxy-8-acetyl coumarin (VII)— 7-Hydroxy-8-acetyl coumarin[19] (5 g.) in dry acetone (200 ml.) was refluxed with anhydrous sodium iodide (4·5 g.), benzyl chloride (3 ml.) and anhydrous potassium carbonate (10 g.) for 8 hr. Acetone solution was filtered and the solvent distilled off and excess of benzyl chloride removed by steam distillation. The solid product (5·4 g.) crystallized from methanol as colourless elongated prisms, m.p. 117-8°. (Found: C, 73·2; H, 5·3. $C_{18}H_{14}O_4$ requires C, 73·5; H, 4·8%.)

Nuclear oxidation — The above coumarin (5 g.) was dissolved in hot aqueous sodium hydroxide (40 ml.; 10 per cent); the solution cooled (15-20°), stirred and treated dropwise with potassium persulphate (6 g. in 150 ml. water) during 2 hr. After 24 hr at room temperature, the deep brown solution was acidified with dilute hydrochloric acid to congo red and the unchanged product filtered off, ether extraction removing the last traces of it. The clear solution was heated in a boiling water bath (20 min.) with sodium sulphite (2 g.) and concentrated hydrochloric acid (75 ml.). The solid on fractional crystallization from methanol gave two products. The more soluble product (6-hydroxy-7-benzyloxy-8-acetyl coumarin, 0·8 g.) on recrystallization from methanol separated as colourless thin rhombic plates, m.p. 190-1°. (Found: C, 69·4; H, 4·9. $C_{18}H_{14}O_5$ requires C, 69·7; H, 4·5%.) The sparingly soluble product (6,7-dihydroxy-8-acetyl coumarin, 0·5 g.) crystallized from methanol as pale yellow rectangular plates and small prisms, m.p. 215-6°. On methylation it gave 6,7-dimethoxy-8-acetyl coumarin[7], m.p. and mixed m.p. 95-6°.

6-Methoxy-7-benzyloxy-8-acetyl coumarin — 6-Hydroxy-7-benzyloxy-8-acetyl coumarin (1 g.) in acetone (100 ml.) was refluxed with dimethyl sulphate (0·5 ml., excess) and anhydrous potassium carbonate (3 g.) for 6 hr. The product (1 g.) crystallized from methanol as colourless stout rhombohedral prisms, m.p. 135-6°. (Found: C, 69·9; H, 5·1. $C_{19}H_{16}O_5$ requires C, 70·4; H, 5·0%.)

6-Methoxy-7-benzyloxy-8-hydroxy coumarin — The above coumarin (1 g.) was dissolved in sodium hydroxide (4 ml.; 4 per cent) and subjected to Dakin's oxidation in the same way as for the preparation of compound (II). The solid product (0·7 g.) crystallized from benzene-petroleum ether mixture as colourless flat needles, m.p. 168-9°. (Found: C, 68·4; H, 5·0. $C_{17}H_{14}O_5$ requires C, 68·5; H, 4·7%.)

6,8-Dimethoxy-7-benzyloxy coumarin — It was prepared by methylation of the above coumarin (0·2 g.) in dry acetone (50 ml.) with dimethyl sulphate (0·1 ml.) and anhydrous potassium carbonate (1 g.) and refluxing for 4 hr. The product (0·2 g.) crystallized from benzene-petroleum ether mixture as colourless rectangular plates and prisms, m.p. 111-3°. (Found: C, 69·1; H, 5·4. $C_{18}H_{16}O_5$ requires C, 69·2; H, 5·2%.)

6,8-Dimethoxy-7-hydroxy coumarin (isofraxidin) (IV) — The above coumarin (0·15 g.) was debenzylated in glacial acetic acid (50 ml.) containing 10 per cent palladium-charcoal catalyst (50 mg.) with hydrogen (15·5 ml. at atmospheric pressure and room temperature). The solvent was filtered off and distilled completely under reduced pressure. The solid product (50 mg.) crystallized from benzene-petroleum ether mixture as colourless stout rectangular prisms, m.p. 147-8°, agreeing with earlier record[2]. (Found: C, 59·1; H, 4·8. $C_{11}H_{10}O_5$ requires C, 59·5; H, 4·5%.)

6-Methoxy-7,8-dihydroxy coumarin (*fraxetin*) (V)— 6-Methoxy-7-benzyloxy-8-hydroxy coumarin (0·16 g.) was debenzylated in glacial acetic acid (50 ml.) containing 10 per cent palladium-charcoal catalyst (50 mg.) with hydrogen (14 ml. at atmospheric pressure and room temperature). The product was worked up as in the above case. It crystallized from benzene as colourless prismatic needles and elongated prisms (60 mg.), m.p. 227-8°, the same as recorded earlier[4].

Its acetate crystallized from methanol as colourless rectangular prisms, m.p. 191-3°, the same as recorded earlier[7]. (Found: C, 57·6; H, 4·2. $C_{10}H_8O_5$ requires C, 57·7; H, 3·9%.)

Dakin's oxidation of 4-methyl-5,7-dimethoxy-8-acetyl coumarin: *4-Methyl-5,6-dihydroxy-7-methoxy coumarin* (X) — 4-Methyl-5,7-dimethoxy-8-acetyl coumarin[8] (IX) (1 g.) was dissolved in aqueous sodium hydroxide

(10 ml.; 4 per cent) and subjected to Dakin's oxidation in the same way as for the preparation of compound (II). The product crystallized from ethyl acetate as colourless rectangular plates, m.p. 255-6°. (Found: C, 59·3; H, 4·7. $C_{11}H_{10}O_5$ requires C, 59·5; H, 4·5%.) Mixed melting point with an authentic sample described below was undepressed. Its ultraviolet spectrum in methanol gave maximum at 315 mμ. It gave a green ferric reaction and on methylation with dimethyl sulphate and anhydrous potassium carbonate in acetone medium yielded 4-methyl-5,6,7-trimethoxy coumarin, m.p. 115-6°, undepressed by an authentic sample. Its ultraviolet spectrum in methanol gave maxima at 310 and 317 mμ.

The acetate was prepared using acetic anhydride and pyridine. It crystallized from ethyl acetate and melted at 168-9°, undepressed by the authentic sample of 4-methyl-5,6-diacetoxy-7-methoxy coumarin described below.

4-Methyl-5-hydroxy-6-acetyl-7-methoxy coumarin (XII) — (a) 4-Methyl-5,7-dihydroxy-6-acetyl coumarin[20] (XI) (2 g.) was refluxed for 6 hr with dimethyl sulphate (0·85 ml.; 1 mole) and potassium carbonate in acetone solution (100 ml.). The solid product (1·6 g.) crystallized from acetic acid as colourless needles, m.p. 256°. (Found: C, 62·5; H, 5·1. $C_{13}H_{12}O_5$ requires C, 62·9; H, 4·9%.) It was insoluble in 10 per cent aqueous sodium carbonate and sparingly soluble in 5 per cent aqueous sodium hydroxide depositing the sodium salt gradually. It gave a pink colour with alcoholic ferric chloride.

(b) To a mixture of 2-methoxy-4,6-dihydroxy acetophenone[21] (2 g.) and ethyl acetoacetate (2 ml.) cooled to 0° was added with stirring concentrated sulphuric acid (20 ml.) slowly at such a rate that the temperature of the reaction mixture did not exceed 5°. The mixture was left for 24 hr at 0° and poured over crushed ice with stirring. The solid that separated crystallized from glacial acetic acid as colourless needles (2·1 g.), m.p. 256°, undepressed by the sample of 4-methyl-5-hydroxy-6-acetyl-7-methoxy coumarin prepared by the method (a) above.

Dakin's oxidation: *4-Methyl-5,6-dihydroxy-7-methoxy coumarin* (X)— The above acetyl coumarin (XII) (1 g.) was treated with aqueous sodium hydroxide (10 ml.; 5 per cent) when the solid first went into solution and then separated as a yellow sodium salt. It was again brought into solution by adding pyridine (25 ml.). The reaction mixture was then cooled (5°) and treated with hydrogen peroxide (1 ml.; 30 per cent) added drop by drop. After 2 hr at room temperature, the reaction mixture was acidified with dilute hydrochloric acid, the solution salted out and extracted with ether. The ether solution was washed with dilute hydrochloric acid and subsequently with water. The solid product obtained after distilling off the solvent crystallized from methanol (0·4 g.), m.p. 255-6°, undepressed by admixture with the sample described earlier.

4-Methyl-5-methoxy-7-hydroxy-8-acetyl coumarin (XIV) — 4-Methyl-5,7-dihydroxy-8-acetyl coumarin[8] (XIII) (3·3 g.) was methylated with dimethyl sulphate (1·4 ml.; 1 mole) and sodium bicarbonate (3 g.) in acetone medium (150 ml.) by refluxing for 6 hr. The product was worked up as usual and the monomethyl ether crystallized from alcohol as colourless prisms (3·0 g.), m.p. 183-4°. (Found: C, 63·4; H, 5·0. $C_{13}H_{12}O_5$ requires C, 62·9; H, 4·8%.) It was soluble in aqueous sodium carbonate but insoluble in aqueous sodium bicarbonate. It gave a pink colour with alcoholic ferric chloride.

On methylation with excess of dimethyl sulphate, and potassium carbonate it gave 4-methyl-5,7-dimethoxy-8-acetyl coumarin, m.p. 178-80°, undepressed by a sample prepared by the method of Sastri *et al.*[8].

On ethylation with diethyl sulphate and potassium carbonate in acetone solution it gave 4-methyl-5-methoxy-7-ethoxy-8-acetyl coumarin, m.p. 164-5°. (Found: C, 65·0; H, 5·7. $C_{15}H_{16}O_5$ requires C, 65·6; H, 5·8%.) It was insoluble in cold sodium hydroxide solution and gave negative ferric reaction.

Dakin's oxidation: *4-Methyl-5-methoxy-7,8-dihydroxy coumarin* (XV)— The above 8-acetyl coumarin (XIV) (2 g.) was dissolved in sodium hydroxide (10 ml.; 4 per cent), the solution cooled to 5° and treated with hydrogen peroxide (2 ml.; 30 per cent). After 2 hr, it was acidified with dilute hydrochloric acid and the solid that separated crystallized from alcohol yielding colourless rectangular prisms (1·5 g.), m.p. 228-30°; Naik and Thakor[22] reported its m.p. as 230-1°. (Found: C, 59·7; H, 4·3. $C_{11}H_{10}O_5$ requires C, 59·4; H, 4·5%.) It was soluble in aqueous sodium carbonate and gave a green ferric reaction. Its ultraviolet spectrum in methanol gave maxima at 272 and 320 mμ.

On methylation with dimethyl sulphate and potassium carbonate in acetone solution, it gave 4-methyl-5,7,8-trimethoxy coumarin, m.p. 173-4°, same as reported by Sastri *et al.*[8]. Its ultraviolet spectrum in methanol gave maxima at 260 and 322 mμ.

Methylenation was effected by boiling with excess of methylene iodide and potassium carbonate in acetone solution for 90 hr. The methylenedioxy derivative crystallized from methanol as colourless flat needles, m.p. 245-6°. (Found: C, 61·0; H, 4·3. $C_{12}H_{10}O_5$ requires C, 61·5; H, 4·1%.) It gave a blue colour with gallic acid and concentrated sulphuric acid and was insoluble in alkali.

Action of alkali on 4-methyl-5,7-dihydroxy-8-acetyl coumarin: 5,7-*Dihydroxy-4-methyl coumarin* — A solution of 4-methyl-5,7-dihydroxy-8-acetyl coumarin in sodium hydroxide (10 ml.; 5 per cent) was heated (100°; for 1·5 hr). The solution was cooled, acidified with dilute hydrochloric acid and the solid that separated crystallized from ethyl acetate, m.p. 283-4°, undepressed by an authentic sample of 4-methyl-5,7-dihydroxy coumarin[23].

4-*Methyl-5,6,7-trihydroxy coumarin*: (*a*) *Dakin's oxidation of 4-methyl-5-methoxy-7-hydroxy-8-acetyl coumarin* — The acetyl coumarin (1 g.) was dissolved in sodium hydroxide (10 ml.; 5 per cent) and the oxidation carried out as in the preparation of compound (II). The product crystallized from ethyl acetate as pale yellow thin plates, m.p. 277-8°, mixed melting point with an authentic sample[8] of 4-methyl-5,6,7-trihydroxy coumarin was undepressed. Its ultraviolet spectrum in methanol gave maxima at 280 and 340 mμ. (4-Methyl-5,7,8-trihydroxy coumarin gave maxima at 258 and 325 mμ.)

(*b*) *Action of alkali on 4-methyl-5,7,8-trihydroxy coumarin* — A solution of the trihydroxy coumarin (0·5 g.) in sodium hydroxide (5 ml.; 5 per cent) was heated (100°) for 30 min., while nitrogen gas was bubbled through the reaction mixture. The product obtained after acidification crystallized from ethyl acetate, m.p. and mixed m.p. with 4-methyl-5,6,7-trihydroxy coumarin was 277-8°.

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Studies in Potential Antimycobacterial Agents: Part XV—Synthesis of Some 2-(2′-Hydroxyphenyl)-5-Alkyl-2-Oxazolines, 2-(2′-Hydroxyphenyl)-2-Thiazolines & Thiazolidines

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Synthesis of some 2-(2′-hydroxyphenyl)-5-alkyl-2-oxazolines, 2-(2′-hydroxyphenyl)-2-thiazolines and thiazolidines as possible antagonists of 2-(2′-hydroxy-6′-methylphenyl)-2-oxazoline — a likely precursor of mycobactin — is described. Oxazolines have been synthesized by cyclization of the appropriate *o*-hydroxy-N-(2-hydroxyalkyl)-benzamides with thionyl chloride. Thiazolines have been obtained by the condensation of β-mercaptoethylamine with appropriate nitriles; while thiazolidines have been prepared by reacting the desired aromatic hydroxyaldehydes with β-mercaptoethylamine hydrochloride. The compounds have been tested *in vivo* against experimental tuberculosis of the guinea-pigs and mice and none of them has been found to be promising.

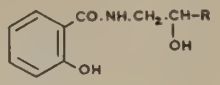
THE observation of Twort and Ingram[1] that *Mycobacterium johnei* could not be cultivated in synthetic media unless dried and killed human tubercle bacilli, or other mycobacteria similarly treated, were added to the culture medium, led to the conclusion that the specific growth substance provided by these dead mycobacterial cells was essential to the metabolism of the whole group of acid fast organisms. This factor was eventually isolated from *M. phlei* and named mycobactin[2]. It has since been shown by Snow[3] to be a derivative of 2-(2′-hydroxy-6′-methylphenyl)-4-carboxyoxazoline. Mycobactin was shown to promote the growth of human tubercle bacilli on media containing horse serum, which do not normally sustain bacterial division[4].

M. tuberculosis has obviously the ability to elaborate its own requirement of mycobactin. It seemed of interest to synthesize analogues of a possible mycobactin precursor which would ultimately block the synthesis of this essential growth factor and thus act as antitubercular agents. As a first step in this study, synthesis of a series of 2-(2′-hydroxyphenyl)-5-alkyl-2-oxazolines, 2-(2′-hydroxyphenyl)-2-thiazolines, with substituents in different positions of the benzene ring, and a few thiazolidines is reported.

The oxazolines were synthesized by cyclization of the appropriate *o*-hydroxy-N-(2-hydroxyalkyl)-benzamides with thionyl chloride according to the method of Pfister *et al.*[5]. The N-alkanol benzamides were prepared from 1-nitro-2-alkanols by reduction with Raney nickel[6] followed by condensation with methyl salicylate. The compounds thus prepared are described in Tables 1 and 2.

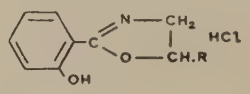
Attempted condensation of salicylthioamide with ethylene dibromide under different experimental conditions[7] did not give the anticipated 2-(2′-hydroxyphenyl)-2-thiazoline. Recourse was, therefore, taken to the method of Kuhn[8] for the synthesis of 2-substituted 2-thiazolines which involved the condensation of β-mercaptoethylamine and appropriate nitriles. The yields of thiazolines were generally satisfactory, *c.* 70-80 per cent, except in the case of 2-(3′-hydroxyphenyl)-2-thiazoline (5 per cent). This reaction, however, completely failed in the condensations involving 6-methyl resorcylonitrile, 2-hydroxy-1-cyanonaphthalene, and 4-nitrosalicylonitrile. Hydroxy benzonitriles were prepared from the appropriate aldehydes by dehydration of the corresponding oximes with acetic anhydride[9]. The thiazolidines were prepared by refluxing the aldehydes with β-mercaptoethylamine hydrochloride in 50 per cent alcohol[10]. The yields of 2-(2′-hydroxyphenyl)-thiazolidines were generally poor (20-40 per cent). 2-(3′-Hydroxyphenyl)- and 2-(4′-hydroxyphenyl)-thiazolidines were obtained in good yields (70-80 per cent).

TABLE 1 — 2-HYDROXY-N-(2′-HYDROXYALKYL)-BENZAMIDES



Sl No.	R	Mol. formula	B.P. °C.	Found % N	Required % N
452	$-CH_3$	$C_{10}H_{13}O_3N$	196-200/4 mm.	7·2	7·2
453	$-C_2H_5$	$C_{11}H_{15}O_3N$	190/3-4 mm.	6·8	6·7
454	n-C_3H_7	$C_{12}H_{17}O_3N$	188/2 mm., m.p. 140-1	5·9	6·3
455	*Iso*-C_3H_7	$C_{12}H_{17}O_3N$	180/2 mm., m.p. 100-1	6·3	6·3
456	n-C_6H_{13}	$C_{15}H_{23}O_3N$	180/3 mm.	5·4	5·3

TABLE 2 — 2-(2′-HYDROXYPHENYL)-5-ALKYL-2-OXAZOLINE HYDROCHLORIDES



Sl No.	R	Mol. formula	M.P. °C.	Found % N	Required % N
457	$-CH_3$	$C_{10}H_{11}O_2N$, HCl	145	6·6	6·6
458	$-C_2H_5$	$C_{11}H_{13}O_2N$, HCl	138	5·9	6·2
459	n-C_3H_7	$C_{12}H_{15}O_2N$, HCl	130	5·9	5·8
460	*Iso*-C_3H_7	$C_{12}H_{15}O_2N$, HCl	153-4	5·9	5·8
461	n-C_6H_{13}	$C_{15}H_{21}O_2N$, HCl	144-5	5·4	4·9

Most of these compounds have been tested *in vivo* against experimental tuberculosis of the guinea-pigs and mice in the Microbiology Division of this Institute. None of these compounds was, however, found to be promising.

Experimental procedure

All melting points and boiling points recorded are uncorrected.

1-*Amino-2-alkanols* — The required 1-nitro-2-alkanols were prepared by the condensation of nitromethane, with the appropriate aldehydes, according to the method of Schmidt *et al.*[11] with slight modifications, viz. omission of extraction with ether after addition of alkali, and use of acetic acid for acidification instead of sulphuric acid. With these modifications yields over 80 per cent were obtained.

These nitro alcohols were reduced to the corresponding 1-amino-2-alkanols by hydrogenation with Raney nickel in an atmosphere of carbon dioxide in *c.* 50 per cent yield[6]. Reduction with ferrous sulphate gave better yields but needed a more laborious working up and the catalytic method was preferred due to its simplicity.

o-Hydroxy-N-(2-hydroxyalkyl)-benzamides — The benzamides were prepared by refluxing a mixture of the alkanolamines (1·5-2 moles) and methyl salicylate (1 mole) on the sand bath for 2 hr, followed by removal of the excess of the amine under vacuum. The benzamides were purified by vacuum distillation and are described in Table 1. The yields of these compounds were *c.* 80-90 per cent.

2-(2′-*Hydroxyphenyl*)-5-*alkyl*-2-*oxazolines* — These were prepared by the following general method. The amide (0·05 ml.) was gradually added under stirring to thionyl chloride (40 ml.) kept at 0° and the mixture allowed to stand for 2 hr at 0°. It was then diluted with excess dry ether (*c.* 400 ml.), and the precipitated oxazoline hydrochlorides filtered, and crystallized from alcohol-ether mixture. The oxazolines thus prepared in *c.* 80 per cent yield are described in Table 2. The ultraviolet spectra of the oxazolines in ethanol at *p*H 5 showed maxima at 242 and 249 mμ (ε, *c.* 8000) and 305-7 mμ (ε, 4500). The free bases were prepared by treatment of a methanolic solution of the hydrochlorides with ammonia, followed by removal of methanol, extraction of the free base with chloroform and vacuum distillation.

6-*Methyl resorcylonitrile* — 6-Methyl resorcylaldehyde (2 g.) dissolved in minimum quantity of ethanol was mixed with hydroxylamine hydrochloride (1·2 g.) in a little water. The mixture was chilled, rendered just alkaline with sodium carbonate solution and left overnight. The reaction mixture was then acidified

with dilute acetic acid, excess of ethanol removed under reduced pressure and the concentrate diluted with water and extracted with ether. Ether was removed and the aldoxime (1·95 g.) crystallized from benzene; m.p. 205°. (Found: C, 57·9; H, 5·7; N, 8·5. $C_8H_9NO_3$ requires C, 57·5; H, 5·4; N, 8·4%.)

The aldoxime (1·9 g.) and acetic anhydride (5 ml.) were refluxed for 2·5 hr, and worked up as usual. The acetylated nitrile so obtained was hydrolysed with potassium hydroxide and the solution acidified with dilute acetic acid and extracted with ether. The ether solution was washed with 2 per cent sodium bicarbonate and the nitrile (1·5 g.) obtained on removal of ether crystallized from aqueous alcohol; m.p. 212°. (Found: C, 64·6; H, 5·0; N, 9·4. $C_8H_7NO_2$ requires C, 64·4; H, 4·7; N, 9·4%.)

5-Phenyl salicylonitrile — 5-Phenyl salicylaldehyde was treated with hydroxylamine hydrochloride in alkaline solution as above. Acidification and subsequent removal of the excess of ethanol gave 5-phenyl salicylaldoxime which was crystallized from benzene; m.p. 134°. (Found: C, 73·3; H, 5·3; N, 6·6. $C_{13}H_{11}NO_2$ requires C, 73·2; H, 5·2; N, 6·6%.)

5-Phenyl salicylonitrile was obtained by treating the aldoxime with acetic anhydride followed by saponification. It crystallized from benzene; m.p. 195°. (Found: C, 79·5; H, 4·9; N, 7·3. $C_{13}H_9NO$ requires C, 80·0; H, 4·6; N, 7·2%.)

2,5-Dihydroxy benzonitrile — Gentisonitrile, obtained by following the procedure of Seebeck[12], was always contaminated with unreacted salicylonitrile. A modified procedure was, therefore, used. The reaction mixture, just acidified with hydrochloric acid, was extracted with ether to remove salicylonitrile. Excess hydrochloric acid was then added followed by heating at 70° for 20 min. Gentisonitrile was extracted with ether, the ether distilled off and the residue crystallized from ether-petroleum ether.

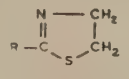
2-(Hydroxyphenyl)-2-thiazolines — 2-(Hydroxyphenyl)-2-thiazolines were synthesized by treating the corresponding nitriles with β-mercaptoethylamine. The process is illustrated by the preparation of 2-(2′-hydroxyphenyl)-2-thiazoline. The various compounds of this series are listed in Table 3. Modifications, wherever necessary, are mentioned.

2-(2′-Hydroxyphenyl)-2-thiazoline (Sl No. 462) — A solution of β-mercaptoethylamine[13] (0·8 g., 0·01 mole) in absolute ethanol (10 ml.) prepared from ethyleneimine[14] (1 ml., 0·8 g.) was freed from H_2S under reduced presure, and refluxed with salicylonitrile (1·2 g.) for 10-12 hr. Ethanol was removed under reduced pressure, water added to the residue and the mixture chilled. The solid thiazoline was filtered and crystallized from petroleum ether (60-80°); yield 1·2 g.; m.p. 52°. (Found: C, 60·8; H, 5·0; N, 7·8. C_9H_9NOS requires C, 60·3; H, 5·0; N, 7·8%.)

In the case of Sl No. 472 (Table 3), the semi-solid obtained by addition of water to the reaction mixture was washed with water and extracted with hot benzene. The solvent was removed and a chloroform solution of the residue passed through an alumina column. Concentration of the chloroform eluate gave the thiazoline (0·1 g.).

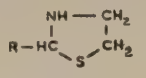
2-(2′-Hydroxy-4′-methoxyphenyl)-2-thiazoline (SlNo. 464) — 2-(2′,4′-Dihydroxyphenyl)-2-thiazoline (0·5 g., 1 mole) in dry acetone (15 ml.) was refluxed with dimethyl sulphate (0·3 ml., 1·2 moles) and anhydrous potassium carbonate (0·7 g., 2 moles) for 8-10 hr and worked up as usual. The resultant product was crystallized from petroleum ether; yield 0·3 g.; m.p. 47°.

TABLE 3 — 2-(HYDROXYPHENYL)-2-THIAZOLINES



Sl No.	R	Mol. formula	Crystallized from	M.P. °C.	Found, %			Required, %		
					C	H	N	C	H	N
462	2-Hydroxyphenyl	C_9H_9NOS	Pet. ether	52	60·8	5·0	7·8	60·3	5·0	7·8
463	2,4-Dihydroxyphenyl	$C_9H_9NO_2S$	Benzene	185	55·8	4·8	7·1	55·4	4·6	7·2
464	2-Hydroxy-4-methoxyphenyl	$C_{10}H_{11}NO_2S$	Pet. ether	47	57·8	5·7	6·4	57·4	5·3	6·7
465	2,4-Dimethoxyphenyl	$C_{11}H_{13}NO_2S$	Purified from pet. ether	Viscous oil	58·8	6·0	6·1	59·2	5·8	6·3
466	2,5-Dihydroxyphenyl	$C_9H_9NO_2S$	Benzene	152	55·5	4·6	7·4	55·4	4·6	7·2
467	2-Hydroxy-5-methoxyphenyl	$C_{10}H_{11}NO_2S$	Pet. ether	58	57·2	5·4	6·5	57·4	5·3	6·7
468	2,5-Dimethoxyphenyl	$C_{11}H_{13}NO_2S$	do	59	59·6	5·5	6·5	59·2	5·8	6·3
469	2-Hydroxy-5-phenylphenyl	$C_{15}H_{13}NOS$	do	82	70·8	5·4	5·6	70·6	5·1	5·5
470	2-Hydroxy-5-chlorophenyl	C_9H_8NOSCl	do	96	50·9	4·0	6·5	50·6	3·7	6·6
471	2-Hydroxy-5-methylphenyl	$C_{10}H_{11}NOS$	do	56	62·4	5·9	7·5	62·2	5·7	7·3
472	3-Hydroxyphenyl	C_9H_9NOS	Benzene	131	60·5	5·3	8·3	60·3	5·0	7·8
473	4-Hydroxyphenyl	C_9H_9NOS	Alcohol	205	60·1	5·5	7·9	60·3	5·0	7·8

TABLE 4 — 2-(HYDROXYPHENYL)-THIAZOLIDINES



Sl No.	R	Mol. formula	Crystallized from	M.P. °C.	Found, %			Required, %		
					C	H	N	C	H	N
474	2-Hydroxyphenyl	$C_9H_{11}NOS$	Benzene-pet. ether	73	59·8	5·7	7·5	59·7	6·1	7·7
475	2-Hydroxy-5-methylphenyl	$C_{10}H_{13}NOS$	Pet. ether	82	61·9	6·4	7·1	61·5	6·7	7·1
476	2-Hydroxy-5-phenylphenyl	$C_{15}H_{15}NOS$	Benzene	164	70·2	5·4	5·4	70·0	5·8	5·4
477	2-Hydroxy-5-chlorophenyl	$C_9H_{10}NOSCl$	Benzene-pet. ether	105	50·4	4·6	6·7	50·1	4·6	6·5
478	3-Hydroxyphenyl	$C_9H_{11}NOS$	Ethanol	167	59·9	6·2	7·7	59·7	6·1	7·7
479	4-Hydroxyphenyl	$C_9H_{11}NOS$	do	190	59·7	6·3	7·7	59·7	6·1	7·7

(Found: C, 57·8; H, 5·7; N, 6·4. $C_{10}H_{11}NO_2S$ requires C, 57·4; H, 5·3; N, 6·7%.)

2-(2′-Hydroxy-5′-methoxyphenyl)-2-thiazoline (Sl No. 467) was also prepared from 2-(2′,5′-dihydroxyphenyl)-2-thiazoline similarly. 2-(2′,4′-Dimethoxyphenyl)-2-thiazoline (Sl No. 465) and 2-(2′,5′-dimethoxyphenyl)-2-thiazoline (Sl No. 468) were also obtained by methylation of the corresponding dihydroxy compounds with 2·5 moles of dimethyl sulphate.

2-(Hydroxyphenyl)-thiazolidines — 2-(Hydroxyphenyl)-thiazolidines were prepared by reacting the desired aromatic hydroxyaldehydes with β-mercaptoethylamine hydrochloride and the method is described under the section on the synthesis of 2-(3′-hydroxyphenyl)-thiazolidine. Compounds of this series are given in Table 4.

2-(3′-Hydroxyphenyl)-thiazolidine (Sl No. 478) — β-Mercaptoethylamine (0·8 g., 0·01 mole), in absolute ethanol (10 ml.), was freed from H_2S as in the preparation of Sl No. 462 and acidified with 20 per cent hydrochloric acid to *p*H 6 with cooling. The reaction mixture was then diluted with water to 20 ml., *m*-hydroxybenzaldehyde (1·2 g., 0·01 mole) added and the mixture refluxed for 1 hr. The excess of alcohol was removed under reduced pressure, the reaction mixture neutralized with sodium bicarbonate to *p*H 9 and chilled. The solid so obtained was filtered, washed with water and crystallized from alcohol; yield 1·5 g.; m.p. 167°. (Found: C, 59·9; H, 6·2; N, 7·7. $C_9H_{11}NOS$ requires C, 59·7; H, 6·1; N, 7·7%.)

In the case of the compound Sl No. 474, it separated as an oil which solidified in a week's time and was crystallized from benzene-petroleum ether; yield 0·5 g.; m.p. 73°.

Compound Sl No. 475 was also obtained as an oil on neutralizing. A sticky solid separated out on keeping it in the refrigerator overnight. This was dissolved in benzene and passed through an alumina column. The thiazolidine (0·1 g.) obtained from the benzene eluate was crystallized from petroleum ether; m.p. 82°.

Crude compound Sl No. 476 was dissolved in benzene containing a little chloroform and the solution chromatographed on alumina. It was eluted first with benzene and then with chloroform. The residue obtained from the combined eluate was crystallized from benzene; yield 0·5 g.; m.p. 164°. Compound Sl No. 477 was worked up in the same manner.

Acknowledgement

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Anthraquinone & Anthrone Series: Part XXIV— Chromatography of Vat Dyes on Alumina at High Temperatures*

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An apparatus and procedure for the chromatographic adsorption analysis of vat dyes on alumina at temperatures between 100° and 200° are described. *o*-Dichlorobenzene, trichlorobenzene, phenol, *o*-chlorophenol and *m*-cresol are useful as solvents for adsorption, development and elution. Model separations are reported.

APPLICATIONS of chromatography to synthetic dyes have been limited in spite of its wide scope. The chief difficulty in the chromatographic analysis of dyes is the limited number of suitable solvents for adsorption, development and elution. A method of purification of direct cotton dyes involving adsorption of an aqueous solution of the dye on a column of powdered cellulose and elution with 80 per cent acetone was described by us in an earlier communication[1].

Vat dyes are insoluble in water, and water-soluble impurities can, therefore, be readily removed. The non-vattable impurities are also readily removed by vatting the dyestuff with alkaline sodium hydrosulphite, filtering and re-oxidizing, but this method should be employed with caution since some dyestuffs thus undergo permanent chemical changes such as dehalogenation and reduction of nitro groups. The vattable portion of a commercial dye may contain a vattable intermediate from which the dye is made, as well as vattable byproducts. One or more vat dyes added for shading purposes may also be present. The insoluble or very sparingly soluble nature of vat dyes in organic solvents has made it difficult to isolate them in pure form. Concentrated sulphuric acid dissolves all vat dyes, but it is unsuitable as a solvent for chromatographic analysis; however, methods involving fractional precipitation or crystallization by careful dilution of the sulphuric acid solution with water, alcohol or acetic acid have been mentioned.

The problem of separation of mixtures of vat dyes has not received adequate attention. Conflicting statements have been made on the separability of alkaline vats on alumina[2-4]. Vat dyes reduced by aqueous caustic soda and sodium hydrosulphite have been chromatographed on columns of bleached sawdust or disintegrated cotton; the chromatogram is then developed in the colours of the oxidized dyes by means of potassium ferricyanide solution[2]. This method appears to be limited to the separation of dyes of widely different substantivity to cellulose.

A generally applicable method for the separation of vat dyes[5] is to vat the mixture of dyes with aqueous tetraethylenepentamine, $NH_2(CH_2CH_2NH)_3CH_2CH_2NH_2$, and sodium hydrosulphite, carry out the adsorption on a column of cellulose powder (80 mesh) and develop the chromatogram with aqueous tetraethylenepentamine containing a little hydrosulphite[6]. One part of the organic solvent and four parts of water were employed both for adsorption and development. A clear and remarkably stable vat is obtained when a vat dye is made into a paste with tetraethylenepentamine and treated with aqueous sodium hydrosulphite at room temperature (20-30°). Employing these conditions several mixtures of vat dyes were separated by Rao *et al.*[5]. *n*-Butylamine, which has been recommended as an effective solvent in conjunction with sodium hydrosulphite for stripping of vat dyes on cotton[7], gives a clear vat in the absence of caustic soda, but it is less effective than tetraethylenepentamine in separating mixtures of vat dyes. One difficulty with this method is the very slow rate of solvent flow; and further, since the leuco compounds of vat dyes have great affinity for cellulose, the movement of zones in the chromatographic column is extremely slow and elution is practically impossible. Addition of pyridine or cellosolve to the

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developing solvent cuts down the affinity of the leuco compounds considerably, and elution of some dyes can then be effected.

Using the vatting solution of Rao *et al.*[5], Klingsberg[8] has studied the chromatographic separation of mixtures of vat dyes on strips of paper and synthetic fibres. Operating in an atmosphere of nitrogen, several mixtures of vat dyes were quantitatively separated, employing spectrophotometric methods for the evaluation of the individual constituents after chromatographic separation. Twenty-two dyes were tabulated in the order of their adsorbability on cellulose, and it was observed that the order of adsorption had no relation to the substantivity of the dyes under normal dyeing conditions. The descending technique was employed for separations, and in the case of strongly held dyes it took days or weeks for separation.

It has been claimed that vat dyes in nitrobenzene solution can be separated on alumina[2], but many vat dyes are insoluble or very sparingly soluble in nitrobenzene in the cold. Bradley and Sutcliffe[9] used trichlorobenzene at 160-70° for the chromatographic isolation of an isoviolanthrone derivative in the pure state. Varhman[10] has isolated the hydrocarbons in primary tars by chromatography in steam-jacketed columns of silica gel and elution with light petroleum (b.p. 120°). Anthracene and chrysene were separated quantitatively from the corresponding quinones on alumina at the boiling point of toluene; the eluate was continuously distilled and the hot vapours passed around the column for heating purposes and subsequently condensed and fed to the top of the column[11].

Lederer[12] has described a vapour-jacketed column suitable for high temperature chromatography. A column maintained at an elevated temperature by means of a jacket containing a self-circulating liquid has been used for hot chromatography by Schram and Primosigh[13], and the apparatus employed in the present work, shown in Fig. 1 and described in the experimental part, is a modification, in which interchangeable ground-glass joints permit columns of different diameters to be used. Thus quantities of alumina varying from 5 to 100 g. can be conveniently handled. Because of the poor solubility of vat dyes in organic solvents in the cold, column chromatography necessitates the use of very large volumes of solvents and becomes impracticable. At higher temperatures most vat dyes have considerably greater solubility, and the present investigation concerns the chromatographic separation of vat dyes at elevated temperatures.

In order to carry out hot chromatography, a column of alumina was built by the wet packing

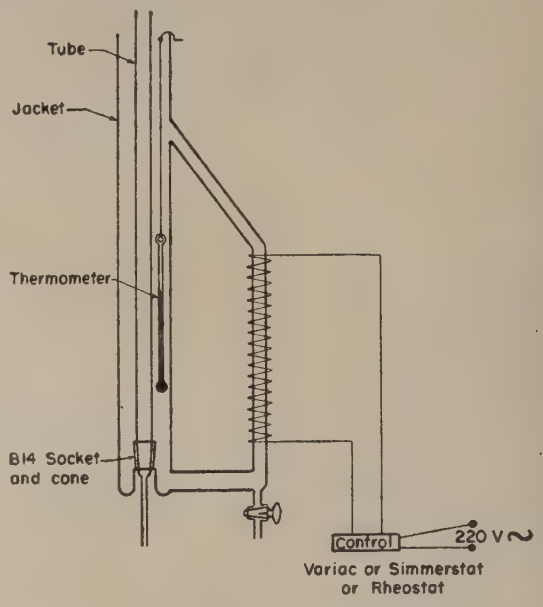

FIG. 1 — APPARATUS FOR HOT CHROMATOGRAPHY

method and maintained at a constant temperature between 100° and 150° (±1°). The dye mixture was added to the column as a hot solution or suspension, and development and elution carried out as usual. The compound crystallized out of the eluate as it cooled down to room temperature, when mere filtration sufficed to isolate the product; or it was recovered by concentration to a small bulk. Following this method several model separations of mixtures of vat dyes were carried out quantitatively. Vat dyes of known constitution were purified by repeated crystallization, their purity checked by elementary analysis and their chromatographic homogeneity determined. Alumina columns maintained at about 110° were used for effecting separations of the mixtures and the individual components recovered in over 95 per cent yield in most cases. The separations were observed visually as differently coloured bands, often with an intervening colourless zone. The isolated fractions were identified by the shades obtained on cotton and by colour reactions.

The solvents generally used for adsorption and development were *o*-dichlorobenzene or 1,2,4-trichlorobenzene, alone or mixed with increasing amounts of phenol or cresols to cut down eluate volumes and elution periods. *o*-Dichlorobenzene containing 3 per cent phenol is a particularly useful solvent in the case of dibenzanthrone derivatives. Molten naphthalene is of interest as a solvent for hot chromatography, but does not offer any distinct advantage over *o*-dichlorobenzene or trichlorobenzene.

The working temperature of the column depends mainly on the solvents employed and the nature of the compounds to be separated. Very high temperatures (150° or above) tend to produce diffuse bands and, therefore, poor separability, while too low a temperature (below 70°) lengthens the time taken for development and elution; a temperature balance is, therefore, struck to combine separability with speed of development. In most cases a temperature of 110° was found to be satisfactory.

Thus isodibenzanthrone separated readily from the isomeric dibenzanthrone; 16,17-dimethoxydibenzanthrone from the dihydroxy compound, the diamino derivative, or the parent quinone; 16-nitrodibenzanthrone from 16-aminodibenzanthrone or the corresponding monobenzamido derivative. The dibenzanthrone derivatives can be arranged in the following order of increasing adsorbability: 16,17-dimethoxy, unsubstituted, 16-nitro, 16-amino and 16-benzamido. The low adsorbability of the dimethoxy compound must be due to the steric interaction of the methoxyl groups[14]. 3,4,9,10-Dibenzopyrene-5,8-quinone and 1,4-*bis*benzamidoanthraquinone were less strongly held on alumina than isodibenzanthrone or dibenzanthrone. Indanthrene Red RK (an acridone) was separated from Indanthrene Yellow FFRK (a carbazole) and from indanthrone, and Indanthrene Red FBB (an oxazole) from indanthrone. Very good separations were observed between flavanthrone and indanthrone or pyranthrone. The separation of flavanthrone from indanthrone is of interest as both compounds are formed during the alkali fusion of 2-aminoanthraquinone at different temperatures.

Experimental procedure

Purification of dyes — The commercial sample was freed from ethanol-soluble and water-soluble matter by extraction with the boiling solvents. The dye was then crystallized from a suitable solvent. Chromatographic purification at high temperature was employed, wherever necessary, and in all cases the chromatographic homogeneity of the purified sample was tested. The elementary analyses of the purified samples are recorded in Table 1.

Solvents — The following solvents were used for carrying out adsorption and development: (1) *o*-dichlorobenzene (ODCB), b.p. 180-2°; (2) 1,2,4-trichlorobenzene (TCB), b.p. 212-3°; (3) phenol, m.p. 41°, b.p. 182°; (4) *m*-cresol, b.p. 203°; and (5) *o*-chlorophenol (OCP), b.p. 176°. All the solvents were dried and distilled before use.

Adsorbent — Alumina (grade I) was used throughout for carrying out the separations.

Description of the apparatus — The chromatographic column (Fig. 1) consists of a pyrex glass tube (2 × 60 cm.) with a standard B14 cone fused near the lower end. The B14 cone of the column rests on a standard B14 socket provided in the jacket which surrounds the column while in use. The jacket, which is 5 cm. in diameter and a few centimetres shorter in length than the column, is also made of pyrex glass tube and has a side arm attached to it as in a Thiele tube. The electrical heating of the self-circulating paraffin jacket is located in the side arm and is controlled. Uniform bath temperature throughout the working length of the column is maintained at any desired point between 30° and 200° by means of a control device (a variac or simmerstat or rheostat) and the temperature is read off from thermometers suitably placed in the jacket. The paraffin can be removed, if necessary, through a tap provided in the side arm.

Operation — The column, plugged with a piece of cotton wool, is kept in place inside the jacket and the bath is heated to about 20° higher than the desired working temperature of the column. The required amount of alumina is made into a thin slurry with the solvent in a beaker and under vigorous mechanical stirring is heated and kept at near-boil for 15-20 min. This pretreatment, which removes moisture and air pockets from the alumina, is important for the satisfactory working of the column, especially when the proposed working temperatures are well above the boiling point of water. The alumina-solvent slurry, which may be cooled if necessary, is poured into the chromatographic column and the adsorbent allowed to settle down uniformly. A small amount of sand may be added to the top of the bed after it has settled down so as to prevent any disturbance during further addition of solvents. The temperature of the bath is finally adjusted to the desired temperature, and adsorption and development carried out as usual. The column may be run overnight without attention, if an adequate supply of developing solvent is provided through a constant level arrangement.

Separation of a mixture of indanthrone and flavanthrone on alumina at 120° — A column of alumina (2 × 30 cm.) was built by the wet method of packing using *o*-dichlorobenzene, and maintained at 120°. A mixture of flavanthrone (20 mg.) and indanthrone (20 mg.) was refluxed with *o*-dichlorobenzene (100 ml.) and the green solution was added to the column while still hot. During adsorption, part of the compound crystallized and settled on the top of the bed as a thin layer. On development with the same solvent at 120°, a lemon-yellow band separated from the strongly held blue zone. The lemon-yellow band was eluted as a lemon-yellow percolate (800 ml.), which, on cooling to room temperature, deposited

TABLE 1 — ELEMENTARY ANALYSIS OF THE PURE DYES USED IN CHROMATOGRAPHIC SEPARATION

Dye and molecular formula	Colour Index No.	Solvent for crystallization	Analysis	Found	Calc.
Indanthrene Red 5 GK (1,4-*bis*benzamido-anthraquinone); $C_{28}H_{18}N_2O_4$	61650	*o*-Dichlorobenzene	C	75·4	75·2
			H	3·8	4·0
			N	6·5	6·3
Indanthrene Yellow FFRK (1,2,7,8-diphthaloylcarbazole); $C_{28}H_{13}NO_4$	69000	Diphenyl oxide	C	78·7	78·7
			H	2·8	3·0
			N	3·3	3·3
Indanthrene Red FBB; $C_{29}H_{14}N_2O_5$	67000	*o*-Dichlorobenzene	C	77·3	77·4
			H	3·1	3·1
			N	6·4	6·2
Indanthrene Red RK (1,2-benzo-5,6-phthaloylacridone); $C_{25}H_{13}NO_3$	68000	do	C	80·3	80·0
			H	3·2	3·5
			N	4·1	3·7
Indanthrone, $C_{28}H_{14}N_2O_4$	69800	*m*-Cresol	C	76·4	76·0
			H	2·8	3·2
			N	6·1	6·3
Pyranthrone, $C_{30}H_{14}O_2$	59700	Diphenyl oxide	C	88·2	88·5
			H	3·4	3·4
3,4,9,10-Dibenzopyrene-5,8-quinone, $C_{24}H_{12}O_2$	—	Nitrobenzene	C	85·9	86·2
			H	3·5	3·6
Dibenzanthrone, $C_{34}H_{16}O_2$	59800	Mixed cresols	C	88·9	89·5
			H	3·8	3·5
16-Nitrodibenzanthrone, $C_{34}H_{15}NO_4$	59850	*o*-Dichlorobenzene	C	81·1	81·4
			H	3·1	3·0
			N	3·0	2·8
16-Aminodibenzanthrone, $C_{34}H_{17}NO_2$	—	do	C	86·1	86·5
			H	3·5	3·6
			N	2·9	3·0
16-Benzamidodibenzanthrone, $C_{41}H_{21}NO_3$	—	do	C	85·3	85·5
			H	3·7	3·7
			N	2·8	2·4
16,17-Diaminodibenzanthrone, $C_{34}H_{18}N_2O_2$	—	do	C	83·1	83·7
			H	3·3	3·7
			N	5·3	5·8
16,17-Dihydroxydibenzanthrone, $C_{34}H_{16}O_4$	—	Pyridine	C	82·7	83·6
			H	2·9	3·3
16,17-Dimethoxydibenzanthrone, $C_{36}H_{20}O_4$	59825	Mixed cresols	C	83·4	83·7
			H	3·5	3·9
			OMe	12·4	12·0
Isodibenzanthrone, $C_{34}H_{16}O_2$	—	*o*-Chlorophenol	C	89·1	89·5
			H	3·7	3·5
Flavanthrone, $C_{28}H_{12}N_2O_2$	70600	Diphenyl oxide	C	82·8	82·5
			H	2·9	2·9

yellow needles. The eluate was concentrated to small bulk (20-30 ml.) and, after cooling, the yellow needles (20 mg.) were isolated and identified as flavanthrone. After discarding the eluate (1·2 litres) from the colourless band, the blue band was eluted as a blue solution (2 litres) out of which part of the dye crystallized on cooling to room temperature. The dye (19·6 mg.) was isolated by concentration to small bulk (30-40 ml.) and cooling, and identified as indanthrone.

Table 2 gives the various model separations carried out, together with experimental data. In all cases

TABLE 2 — CHROMATOGRAPHIC SEPARATIONS OF VAT DYES AT HIGH TEMPERATURES

No.	Components of mixture in order of elution	Weight in mg. Present	Found	Colour of band	Solvent	Temperature °C.	Remarks on separation
1	Flavanthrone	20	20·0	Yellow	ODCB	120	Excellent
	Indanthrone	20	19·6	Blue	ODCB	—	
2	Flavanthrone	10	9·6	Yellow	ODCB	120	Good
	Pyranthrone	10	9·2	Orange	ODCB	120	
3	Indanthrene Red RK	20	19·2	Red	ODCB	120	Excellent
	Indanthrone	20	19·4	Blue	ODCB	120	
4	Indanthrene Red FBB	20	19·8	Red	ODCB	110	do
	Indanthrone	20	19·6	Blue	ODCB	110	
5	3,4,9,10-Dibenzpyrene-5,8-quinone	20	20·0	Red	ODCB	110	do
	Indanthrone	20	19·4	Blue	ODCB	110	
6	Indanthrene Red RK	10	9·6	Red	ODCB	110	do
	Indanthrene Yellow FFRK	10	9·4	Yellow	ODCB	110	
7	Isodibenzanthrone	25	20·0	Violet	TCB+3% cresol	120	Fair
	Dibenzanthrone	25	15·0	Blue	do	140	
8	1,4-*bis*Benzamidoanthraquinone	20	19·6	Red	ODCB	110	Excellent
	Isodibenzanthrone	20	17·4	Violet	ODCB+3% phenol	120	
9	3,4,9,10-Dibenzopyrene-5,8-quinone	10	10·0	Red	ODCB	110	do
	Isodibenzanthrone	10	8·0	Violet	ODCB+3% phenol	120	Fair
	Dibenzanthrone	10	7·2	Blue	TCB+3% cresol	140	
10	16,17-Dimethoxydibenzanthrone	20	18·4	Blue-green	ODCB+1% phenol	110	Excellent
	Dibenzanthrone	20	15·0	Blue	TCB+3% cresol	140	
11	16,17-Dimethoxydibenzanthrone	20	18·8	Blue-green	ODCB+1% phenol	110	do
	Isodibenzanthrone	20	17·0	Violet	ODCB+3% phenol	120	
12	16,17-Dimethoxydibenzanthrone	10	9·4	Blue-green	ODCB+1% phenol	110	do
	16,17-Dihydroxydibenzanthrone	10	—	Green	Band was not eluted out		
13	16,17-Dimethoxydibenzanthrone	15	14·6	Blue-green	ODCB+1% phenol	110	do
	16,17-Diaminodibenzanthrone	15	13·6	Green	ODCB+3% phenol	120	
14	16-Nitrodibenzanthrone	10	9·0	Violet	do	110	Good
	16-Aminodibenzanthrone	10	9·0	Green	do	110	
15	16-Aminodibenzanthrone	10	9·2	do	do	110	do
	16-Benzamidodibenzanthrone	10	8·6	Violet	do	110	

Excellent: a clear colourless zone between bands; good: no interval, but complete separation; fair: slight overlapping of bands; ODCB: *o*-dichlorobenzene; and TCB: 1,2,4-trichlorobenzene.

alumina columns (2 × 30 cm.) slurried with *o*-dichlorobenzene were employed.

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Letters to the Editor

SOME LUMINESCENCE PHENOMENA IN URANYL NITRATE

Scintillation and luminescence phenomena in uranyl nitrate hexahydrate, investigated by keeping a single crystal or mass of crystals in tubes under low pressure, appear to be connected with the pyroelectric property of the salt. Interesting differences have been observed in the characteristic luminescence and fluorescence spectra of powdered samples and crystals of uranyl nitrate under pressure, and of uranyl nitrate hexahydrate and hexadeuterate and their slowly cooled solutions.

CRYSTALS OF URANYL NITRATE HEXAHYDRATE SHOW flashes of luminescence on being powdered[1,2] or dropped into liquid air[3]. Both these phenomena are described as tribo-luminescence, and emission of light in the latter case is also attributed to cracking of crystals on cooling. Since these crystals attract ice particles[4] from liquid air and the luminescence in all probability is due to development of electric charges in the crystal, these phenomena were studied by keeping a single crystal or mass of crystals in evacuated tubes (pressure 10^{-3} mm.) containing air or other gases. On cooling, flashes of luminescence and discharge occurred inside the tubes for *c.* 15-20 min. till the whole mass attained the constant temperature of liquid air. The cooled crystal on warming showed brilliant flashes and a faint after-glow lasting for a few seconds for each discharge. Slow cooling or warming of a single crystal gives scintillations without showing noticeable cracks even after repeating the process several times. Other conditions remaining unchanged, the crystals enclosed in a wire-gauge do not show either luminescence or electrical discharge on cooling or warming. The occurrence of luminescence with change of temperature thus appears to be connected with the pyroelectric property[5] of the substance.

Uranyl nitrate, when powdered, shows little tribo- or pyro-luminescence. Fine powders can be prepared (i) by mechanical crushing, (ii) by slow evaporation of highly acidic solution or (iii) by precipitating out

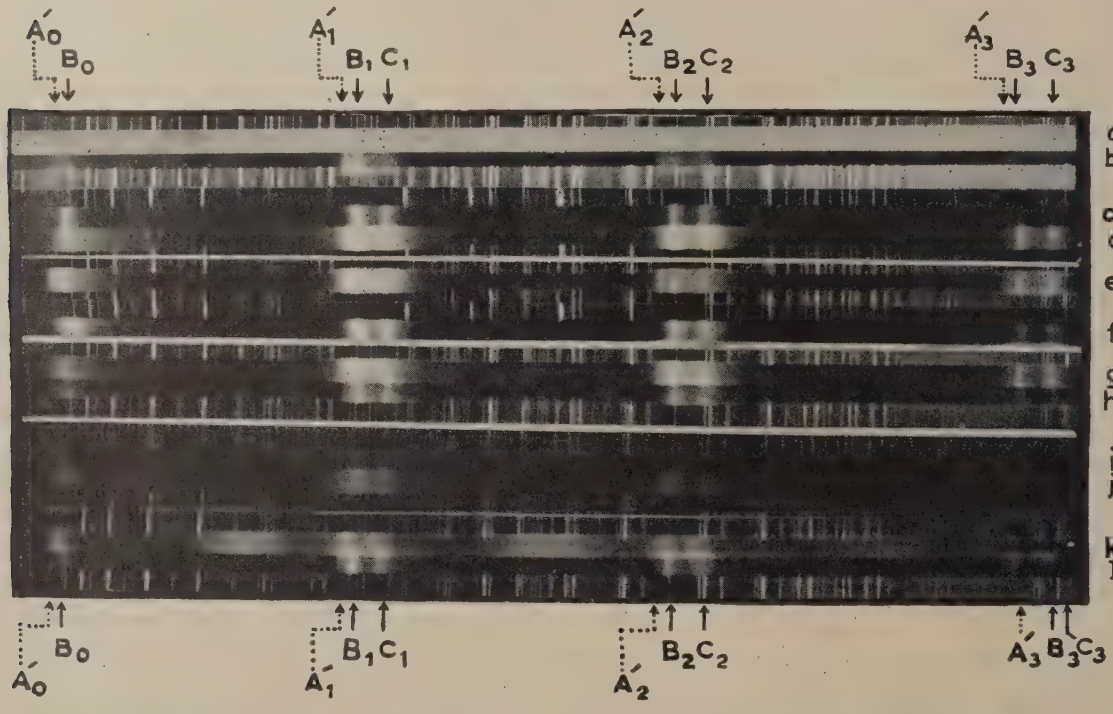

FIG. 1 — SPECTRA OF URANYL NITRATE AT LIQUID AIR TEMPERATURE [In absorption: a, a single crystal of $UO_2(NO_3)_2.6H_2O$ under pressure. In fluorescence: b, same crystal of $UO_2(NO_3)_2.6H_2O$ under pressure; c, same crystal after powdering; d, without pressure but with the same orientation as in 'b'; e, $UO_2(NO_3)_2.6D_2O$ under pressure; f, $UO_2(NO_3)_2.6D_2O$ powdered; g, $UO_2(NO_3)_2.6D_2O$ crystal without pressure; h, $UO_2(NO_3)_2.6H_2O$ crystal without pressure; i, hexahydrate $2M$ aqueous solution (slowly cooled); j, hexahydrate crystals without pressure; k, hexadeuterate $2M$ solution in D_2O; and l, hexahydrate crystals without pressure]

the solid from a very concentrated solution by sudden cooling to liquid air temperature. The spectra of these powders show an interesting change both in absorption and fluorescence (on ultraviolet excitation) (Fig. 1). While the main series of bands (B and C) appear both in powders and crystals without any change in their positions, a series of bands[6] (A'), 40 cm.$^{-1}$ to the violet of each B band, disappears in all powdered samples. The intensity of this A' series increases if the crystal is pressed between two plates and even powders show this violet companion series weakly if subjected to pressure. A' series appears more prominently in absorption, and shows an isotopic shift of 8 cm.$^{-1}$ like the B series on D_2O substitution. In the powders as well as in slowly cooled solutions, two very faint bands appear to the violet side of B bands at 26 and 52 cm.$^{-1}$ respectively, and the A' series of bands disappears. How far the peculiar behaviour of the A' series of bands is connected with strain in the crystal or tribo-luminescence is not clear at present. Further work is in progress.

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STUDIES ON VANADIUM OXIDE CATALYST IN THE OXIDATION OF ANTHRACENE TO ANTHRAQUINONE

An attempt has been made to correlate the yield of anthraquinone from anthracene with the changes in the crystal phase composition of the V_2O_5-Fe_2O_3-K_2SO_4-SiO_2 gel catalyst under different reaction conditions. X-ray diffraction study of the catalyst heated to 900°C. shows that silica gel and iron oxide get converted to α-cristobalite and haematite respectively with no clear evidence for vanadium oxide in the crystalline state. The crystal phase composition of the catalyst remains practically unchanged even after 70 hours' use indicating its long active life.

Anthraquinone is an important intermediate in the manufacture of vat, wool, mordant and cellulose acetate dyes. Investigations were, therefore, undertaken at this Institute with a view to exploring the possibilities of producing this chemical from the technical grade anthracene obtained from coal-tar. The catalytic vapour phase oxidation of anthracene using vanadium oxide catalyst in a fluidized bed has been studied and the results are presented in this note. An attempt has also been made to correlate the percentage yield of anthraquinone from anthracene with the changes in the crystal-phase composition of the catalyst under different reaction conditions. An X-ray study of the catalyst after different thermal treatments and at different stages of reaction has, therefore, been carried out.

The catalyst employed in the fluidized bed was of 60-100 mesh (B.S.S.) size and had the following chemical composition: SiO_2, 85; Fe_2O_3, 6·0; V_2O_5, 6·0; and K_2SO_4, 3·0 weight per cent. The vapours of anthracene, mixed with air, were passed over the catalyst at about 450°C. The oxidation products, collected in air-cooled condensers, were analysed for phthalic anhydride, maleic anhydride, anthraquinone, unchanged anthracene, etc. From the results given in Table 1 it can be observed that the yield of anthraquinone on the basis of anthracene reacted is about 84 per cent.

The following samples of the catalyst were taken for the X-ray study: sample 1, catalyst as prepared; sample 2, catalyst after initial heating at 500°C. for 2 hr; sample 3, sample 2 after initial heating at 900°C. for 1 hr; sample 4, sample 3 after 60 hr run; and sample 5, sample 3 after 70 hr run.

X-ray data for the catalyst samples presented in Table 2 and Fig. 1 reveal some interesting facts. The number of lines in the case of samples 1 and 2 are few, and their relative intensities are weak and diffuse, while a large number of lines are recorded in the case of samples 3, 4 and 5. The diffuse band of silica gel with 'd' value 4· 20 A. is present in the X-ray picture of samples 1 and 2; besides this, there is an indication of the presence of a few weak and

TABLE 1 — YIELD OF ANTHRAQUINONE WITH DIFFERENT FORMS OF CATALYST

Catalyst sample	Space velocity of feed* *g./hr/litre catalyst*	Space velocity of air* *litre/hr/litre at N.T.P. catalyst*	Catalyst temp. °C.	Yield of anthraquinone %	Anthraquinone on anthracene reacted %
2	20·98	4400	400	9·98	9·98
3	21·84	4400	450	72·31	83·52
4	24·36	4400	450	71·43	81·47
5	19·62	4400	450	69·88	83·92

*Results are calculated for 1 litre of catalyst in the static state.

TABLE 2 — X-RAY DATA OF VANADIUM-IRON OXIDE CATALYST

Catalyst sample 1		Catalyst sample 2		Catalyst sample 3		Catalyst sample 4		Catalyst sample 5		α-Cristobalite (A.S.T.M. Card No. 4-0379)		α-Fe_2O_3 (haematite) (A.S.T.M. Card No. 5-0637)	
Intensity	'd' value	Intensity	'd' value	Intensity	'd' value	Intensity	'd' value	Intensity	'd' value	Intensity	'd' value	Intensity	'd' value
m−	9·500	s(band)	4·200	w−	5·010	bvs	4·075	vs	4·040	100	4·0400	30	3·690
m−	8·430	dm	3·350	bs	4·046	w−	3·630	w−−	3·620	12	3·1380	100	2·710
w+	6·420	w	2·822	vw	3·660	w−	3·410	vvw	3·400	14	2·8450	50	2·520
w+	5·083	w	2·392	w+	3·240	w++	3·135	m−	3·145	18	2·4890	25	2·210
vs(band)	4·200	w−	1·841	w+	3·145	w++	2·839	m−−	2·835	4	2·1210	30	1·840
w+	3·366			vvw	2·990	w+	2·682	m−−	2·675	3	2·0240	40	1·690
bm+	3·060			m−	2·848	m+	2·472	s	2·482	4	1·9320	12	1·600
w+	2·857			m−−	2·695	vvw	2·358	w−−	2·362	4	1·8740	30	1·481
w−	2·521			m+	2·485	w−−	2·189	w−	2·194	1	1·7560	30	1·449
w−	2·410			vvw	2·376	w	2·106	w	2·113	1	1·7360	12	1·256
w+	2·295			w−	2·199	w	2·014	w	2·016	3	1·6920	12	1·188
w−−	2·094			w	2·118	w+	1·930	w+	1·930	<1	1·6420	12	1·160
w−	1·983			w	2·025	w+	1·866	w	1·868	5	1·6120	20	1·139
w	1·829			w	1·935	w	1·828	w−	1·831	<1	1·5740	20	1·101
				w	1·875	w++	1·686	vvw	1·750	2	1·5350	30	1·054
				w−	1·836	w++	1·605	w++	1·691	3	1·4950	10	1·037
				w+	1·690	w	1·529	w++	1·608	2	1·4320	10	0·988
				w+	1·608	w+	1·485	w	1·533	1	1·4230		
				dvw	1·535	w−	1·445	w+	1·488	1	1·4010		
				w	1·489	w	1·428	w−	1·449	1	1·3680		
				w	1·451	vw	1·397	w	1·428	1	1·3530		
				w	1·431	w	1·366	vw	1·399	1	1·3450		
				w	1·403	w	1·346	w	1·367	1	1·3360		
				vvw	1·370	vvw	1·304	w	1·346	2	1·3010		
				w	1·348	w	1·280	w	1·302	2	1·2820		
				w	1·306	vw	1·221	w	1·280	3	1·2250		
				w	1·283	vw	1·206	vvw	1·259	1	1·2070		
				w	1·227	w+	1·186	w−	1·234	2	1·1842		
				w	1·206	w−	1·161	w−	1·225	1	1·1762		
				w	1·189	w−	1·140	w−	1·205	1	1·1659		
				vw	1·167	w	1·098	w	1·185	<1	1·1112		
				vw	1·143	w−	1·058	w−	1·167	3	1·0989		
				w	1·098	w−	0·909	w−	1·139				
						w−	0·957	w+	1·099				

s, strong; m, medium; w, weak; bm, broad and medium; bs, broad and strong; dm, diffuse and medium; vw, very weak; bvw, broad and very weak; dvw, diffuse and very weak; vvw, very very weak.

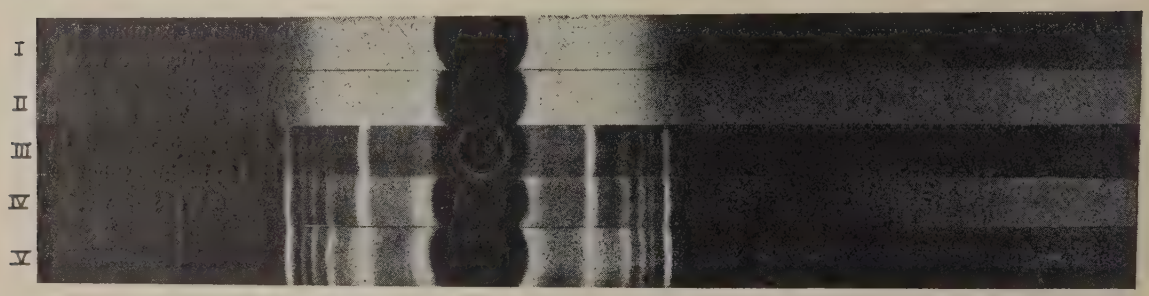

FIG. 1 — X-RAY PHOTOGRAPHS OF VANADIUM-IRON OXIDE CATALYST [I: sample 1, $FeSO_4(NH_4)_2SO_4.6H_2O$ identified as a minor phase; II: sample 2, $V_{12}O_{26}$ identified as a minor phase; III: sample 3, identified as α-cristobalite and haematite; IV: sample 4, identified as α-cristobalite and haematite; and V: sample 5, identified as α-cristobalite and haematite]

diffuse lines corresponding to the strongest lines due to $FeSO_4(NH_4)_2SO_4.6H_2O$ and $V_{12}O_{26}$ respectively in the case of samples 1 and 2. Lines due to α-cristobalite and α-Fe_2O_3 have been identified in samples 3, 4 and 5. However, no evidence has been found for the presence of vanadium oxide in the crystalline state. It is probable that the vanadium compound exists in a fine state of division in the composition of the catalyst.

It is clear from the data presented in Table 1 that the catalyst heated to 500°C. (sample 2) has more drastic oxidizing properties and gives only about 10 per cent yield of anthraquinone, the rest of the feed being mostly oxidized to CO_2 and H_2O. However,

on heating the catalyst to 900°C. (sample 3), the drastic oxidizing nature is modified considerably, and oxidation becomes selective resulting in preferential promotion of the conversion reaction to anthraquinone. X-ray study of this catalyst (sample 3) shows the presence of crystalline phases like α-cristobalite and α-F_2O_3 in the composition of the catalyst. However, the crystal phase composition of this catalyst remains practically unchanged even after use for 70 hr. This probably accounts for the long active life of the catalyst.

Thanks are due to Dr A. Lahiri, Director, and to Shri A. N. Basu, Assistant Director, Central Fuel Research Institute, for their keen interest in this study.

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ETCH FIGURES ON CLEAVAGE PLANE OF BISMUTH

Single crystals of bismuth, grown from the melt by Bridgmann's method after cleavage along the (111) plane and chemically polished, exhibit at some places characteristic triangular patterns consisting of edges made up of broken triangles. The specimen on subsequent etching in 5 per cent aqueous solution of silver nitrate shows triangular etch pits with random distribution. Rows of closely packed triangular etch pits are also observed which may represent the dislocation sites.

In this laboratory a systematic optical study of etch phenomena on natural and synthetic crystal surfaces has been undertaken and as a part of the programme, studies on metal crystals is also carried out. Observations on the etching of single crystals of bismuth (99·9 per cent pure) are reported in this communication.

Dislocation etch pits have been reported[1] on (111) cleavage plane of bismuth. In our laboratory single crystals of bismuth were grown from the melt by Bridgmann's method using the gradient furnace. The crystals were cooled to liquid air temperature and cleaved with a sharp blade along the (111) plane. The two surfaces obtained by cleaving were studied using high resolution microscopy and multiple beam interferometry. The cleaved surface was polished chemically according to Lovell and Wernick[1] and the surface examined under microscope. In some of the specimens characteristic triangular patterns consisting of edges made up of broken triangles were observed after polishing the specimen for about 2 min. (Fig. 1A). In some specimens very few large triangular etch pits were found scattered at random on the surface. Probably these pits may be due to deep imperfections, extending to greater depths within the surface.

A polished specimen was subsequently etched for about 5 sec. with 5 per cent aqueous solution of silver nitrate. Triangular etch pits with a random distribution were observed in the specimen. Rows of closely packed triangular etch pits were also found in some specimens (Fig. 1B). These rows can be attributed to dislocations and it is proposed to study this in greater detail by straining or annealing the specimen. On etching for a further period the triangular etch pits became deeper and gave the appearance of familiar block patterns as observed on diamond by Omar *et al.*[2] and on fluorite by Pandya and Pandya[3] (Fig. 1C).

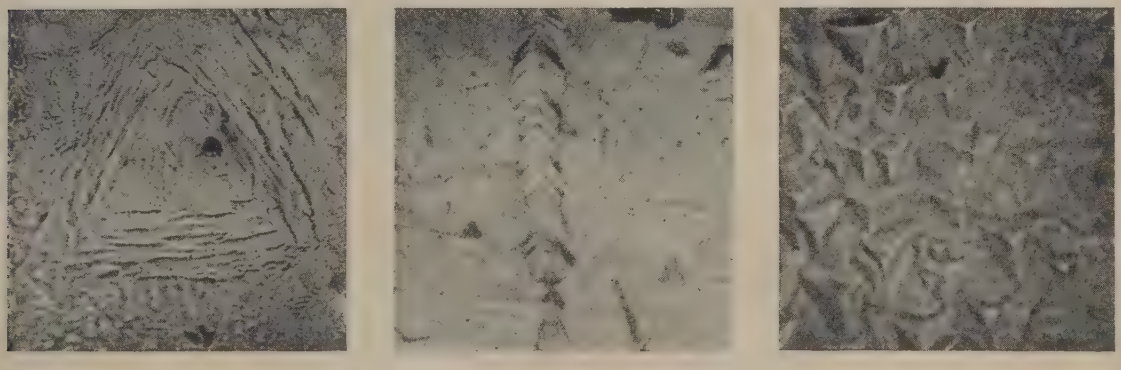

Fig. 1 — Etch figures on the cleavage plane (111) of bismuth single crystal [A: characteristic triangular pattern at deep imperfection revealed on chemical polishing × 340; B: rows of triangular etch pits obtained on etching with silver nitrate solution × 1500; and C: advanced stage of etching × 1500]

To study the etch figures on polycrystals, bismuth was melted in a crucible and poured on a hot glass plate, the temperature of which was kept slightly above the melting point of bismuth. It was then allowed to cool slowly and the specimen was detached from the glass plate by breaking the latter with a diamond cutter according to the technique of Tolansky and Nickols[4]. The surface of the specimen thus obtained was highly smooth and polished. On etching the surface it was found that most of the specimens exhibited surfaces similar to those of single crystals.

On etching the surface with 5 per cent aqueous solution of silver nitrate sharp triangular etch pits distributed at random were obtained in certain grains. This suggests that the surfaces obtained on casting usually belong to the (111) plane. Further work is in progress and the details will be published elsewhere.

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DOUBLE SULPHATE OF IRON (II) & TITANIUM (IV)

A crystalline double salt of ferrous and titanyl sulphates, prepared for the first time, is reported. The product is a dirty yellow, hygroscopic powder, with composition approximating to $3FeSO_4.TiOSO_4.5H_2O$.

No double sulphate of iron (II) and titanium (IV) has so far been reported in literature. We have, however, found that a double salt with a mole ratio of Fe : Ti = 3 : 1 can be easily prepared by the following method.

Fifty ml. (= 0·172 mole of Ti) of titanium (IV) sulphate solution, containing 275 g./litre of TiO_2 and 458 g./litre of H_2SO_4, were heated on a water bath. Twenty-four g. (= 0·086 mole Fe) of ferrous sulphate heptahydrate and 0·2 g. iron wire (in small bits) were added to the titanium solution and the solution stirred. It was then quickly evaporated to a sp. gr. of 1·50 (time about 5 min.). After cooling, the solution was mixed with half its volume of concentrated sulphuric acid, heated for a minute at 160°C., and then allowed to stand in a closed flask for about a week. The crystals were centrifuged and washed with 95 per cent alcohol and finally with absolute alcohol. They were then pressed between folds of filter paper and kept in a desiccator over anhydrous calcium chloride. The product was a fine pale dirty yellow, hygroscopic powder which on analysis gave a mole ratio Fe: Ti = 3: 1 (although the water of hydration was variable). Very little Fe (III) was present in the sample. A typical analysis gave Fe, 22·8; Ti, 6·5; SO_4, 55·2 per cent; $3FeSO_4.TiOSO_4.5H_2O$ requires Fe, 23·8; Ti, 6·8; SO_4, 54·4 per cent.

These investigations show that iron (II) and titanium (IV) sulphates form at least one double salt of the type $3FeSO_4.TiOSO_4.xH_2O$. Also, a mixed sulphate solution of iron (II) and titanium (IV) shows rather strong absorption in the visible region of the spectrum below 500 mμ. Since solutions of $FeSO_4$ or $TiOSO_4$ show relatively little absorption in this region, we think that a fair degree of complex formation takes place between these salts.

We thank Dr J. Gupta for his keen interest in the above investigation.

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THERMOGRAVIMETRIC BEHAVIOUR OF CEROUS OXALATE

The thermogravimetric behaviour of cerous oxalate with varying amounts of water of hydration have been investigated making use of a quartz fibre spring. The thermogravimetric curves prepared indicate different stages of hydration and decomposition of the oxalates. The lowest hydrate that could be prepared as a result of the thermogravimetric analysis corresponds to the composition $[Ce_2(C_2O_4)_3O.5H_2O]$; this is obtained by heating the decahydrate in carbon dioxide atmosphere at 200-220°C. It is impossible to get pure anhydrous cerous oxalate as it is not possible to remove the last traces of water without decomposing the oxalate.

Conflicting results have been reported in literature about the thermal decomposition of cerium and other rare earth oxalates by several workers[1-6]. According to Duval[1] thermogravimetric curves are not well defined but indicate only continuous drop in weight up to 450°C. and do not distinguish between different stages of decomposition, viz. the removal of water and the evolution of carbon monoxide and carbon dioxide. Wendlandt[6] has reported that water of hydration is evolved at 50°C.

and a break appears only at 205°C. Above 300°C., however, the decomposition becomes rapid yielding finally ceric oxide at about 360°C. Preliminary experiments carried out by the present authors indicated that the rate of heating has considerable influence on the different stages of decomposition of the oxalate and a detailed investigation on the thermogravimetric behaviour of cerous oxalate was undertaken in view of its importance in the chemistry of rare earths.

Preparation of different hydrated cerous oxalates — The ceric oxide employed for the preparation of oxalates hydrated to different extents was extracted from the rare earth chloride residues from the Indian Rare Earth Company, Alwaye (India), which were free from thorium and phosphates. The product was purified by standard methods[7].

For the preparation of cerous oxalate decahydrate, $[Ce_2(C_2O_4)_3.10H_2O]$, the ceric oxide was dissolved in concentrated nitric acid and diluted with water and filtered. The filtrate was heated to boiling and the cerous oxalate was precipitated by the slow addition of a hot solution of 10 g. A.R. oxalic acid in 50 ml. of water. The mixture was digested on a water bath for 20 min. and filtered. The precipitate was washed thoroughly to remove free oxalic acid. Final washings were made with ethyl alcohol and then with ether. A current of dry air was drawn to dry the precipitate.

The product thus obtained was analysed for its cerium and oxalate contents by the standard methods[8]. The water content was determined by heating a weighed sample of the substance to 500°C. in a current of dry oxygen and the moisture evolved was absorbed by anhydrous magnesium perchlorate and weighed[9]. The results of the analysis indicated that the hydrated oxalate corresponded to the formula $[Ce_2(C_2O_4)_3.10H_2O]$. It has to be pointed out, however, that the water content varied slightly between 9·8 and 10·2 moles.

Cerous oxalate is known to contain varying amounts of water of crystallization depending upon the conditions of precipitation and mode of drying. It is essential, therefore, to carry out the preparation under controlled experimental conditions in order to obtain a product of uniform composition.

Cerous oxalate with nine molecules of water of crystallization $[Ce_2(C_2O_4)_3.9H_2O]$ was prepared by washing the decahydrate with hot water (80°C.).

Cerous oxalate with two molecules of water of hydration was prepared by drying the deca- or nonahydrate at 120°C. for 12-24 hr. The analysis of this partially hydrated oxalate sample indicated that the water of hydration varied from 2·1 to 2·5 moles. Cerous oxalate hemihydrate[5] $[Ce_2(C_2O_4)_3]_2.H_2O$ was prepared by heating the decahydrate at 200-220°C. in an atmosphere of carbon dioxide for 2-3 hr. Analysis of this sample for cerium, oxalate and water contents confirmed the above composition.

Thermogravimetric behaviour of all these samples of hydrated cerous oxalate were investigated as described below.

Thermogravimetric balance — The apparatus used for the study of the thermogravimetric behaviour of the cerous oxalate consisted essentially of a quartz fibre spring fitted with suitable quartz bucket and housed in a jacket and introduced in a furnace as described earlier[10]. The rate of heating of the furnace was controlled by a variac. The temperature of the furnace was measured with the help of a calibrated chromel-alumel thermocouple situated quite close to the suspended bucket. The elongation or contraction of the quartz spring with reference to a rod suspended at the upper hook was measured with the help of a cathetometer which could read correct to 0·01 mm. The sensitivity of the spring was 30 mm. for a load of 100 mg.

A known amount of cerium oxalate (about 100 mg.) was taken in the quartz bucket (tared), suspended from the quartz fibre spring and introduced into the jacket and housed in the furnace. The furnace was heated gradually at about 2°C./min. (or any other required rate).

The loss in weight of the substance was followed with the help of the cathetometer. Constant temperature was maintained whenever it was necessary for a longer interval (100-120 min.) by means of the variac. An inert atmosphere was maintained whenever it was required by passing a stream of nitrogen through a jet attached at the bottom of the jacket. Appropriate ventilation arrangements were made for the escape of the hot gases in the upper part of the jacket. The loss in weight suffered by the sample was registered and plotted against the corresponding temperatures and the thermogravimetric curves were drawn.

Thermal decomposition of cerous oxalates — The thermogravimetric curve 1 in Fig. 1 refers to the behaviour of cerous oxalate with ten molecules of water of hydration when it was continuously heated at the rate of 2°C./min. It can be made out from the graph that the crystals begin to give off water at 80°C. and the loss in weight continuous till 360°C. Different stages can easily be demarcated in the curve at a, b, c, d, e and f. In the beginning the loss in weight is found to be small (ab) but at 120°C. the fall in weight is steep and this continues till 200°C. is reached. There is a short horizontal stage between 200° and 220°C. (cd). At this stage loss in weight corresponds to 24 per cent of the original weight of the substance taken for the experiment. If the

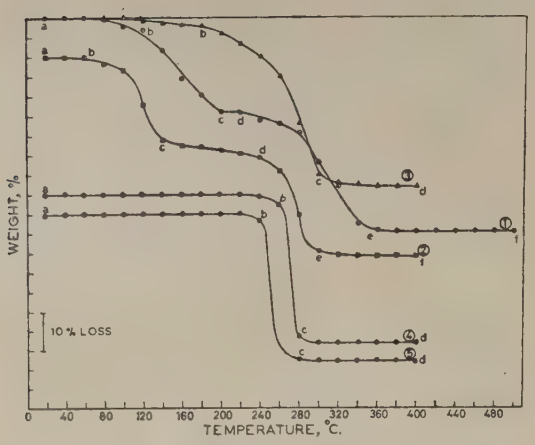

FIG. 1 — THERMOGRAVIMETRIC CURVES FOR CEROUS OXALATE HYDRATED TO DIFFERENT EXTENTS [Curve 1 : $Ce_2(C_2O_4)_3.10H_2O$; curve 2: $Ce_2(C_2O_4)_3.9H_2O$; curve 3: $Ce_2(C_2O_4)_3.2{\cdot}5H_2O$; curve 4: $Ce_2(C_2O_4)_3O.5H_2O$ (all in air); and curve 5: $Ce_2(C_2O_4)_3.0{\cdot}5H_2O$ (in nitrogen)]

entire quantity of water associated with the crystals were to be expelled the loss in weight should correspond to 24·88 per cent of the decahydrate. Therefore, it can easily be made out that most of the water (more than 96 per cent) is expelled at this stage when the sample is taken to 200-220°C. The composition of the residual material left in the bucket could be computed from the weight loss and this corresponds to a hemihydrate [$Ce_2(C_2O_4)_3O.5H_2O$]. (This observation was confirmed on analysis of a sample prepared under identical conditions in a separate experiment for its cerium, oxalate and water contents.) With further increase in temperature the sample suffered further loss in weight during the interval 220-340°C. (de). Thereafter the weight remained constant between 360° and 500°C. The final product of decomposition was found by analysis to be ceric oxide (CeO_2).

A similar behaviour was noticed in the case of cerous oxalate containing nine molecules of water of hydration.

If the temperature of the bucket is maintained between 120° and 140°C. for a longer interval (*c.* 120 min.), far greater amount of water is lost than as indicated by the thermogravimetric curve 1. Nearly 90 per cent of the total water content is removed in this range. When the temperature was raised after this interval, the fall in weight was correspondingly lower till a temperature of 240°C. was reached in the course of a period of about 150 min. The decomposition was found to be rapid as in earlier case beyond 240°C. and reached a constant value after 340°C. The final residue, left behind, corresponded to ceric oxide. The thermogravimetric curve 2 in Fig. 1 corresponds to this experiment.

It is clear from the above observations that if cerous oxalate with either nine or ten molecules of water of hydration is maintained between 120° and 140°C. for a longer interval (2-3 hr), major portion of the water is lost. There is very little further loss in weight even if the sample is maintained at this temperature for 3 hr or more. This was again confirmed by a separate experiment. About 2·5 g. of cerous oxalate nonahydrate was kept at 120°C. for more than 16 hr. The crystals lost 16·7 per cent of the original weight corresponding to 72·7 per cent of water. The formula of this sample corresponded between di- and trihydrate [$Ce_2(C_2O_4)_3.2{\cdot}5H_2O$].

Thermogravimetric behaviour of the above lower hydrate of cerous oxalate is indicated in curve 3 (Fig. 1). It is seen from the graph that there is very little loss in weight of the sample up to 160°C. and thereafter the fall in weight is continuous up to 320°C. The fall in weight is steeper in the later range (260-320°C.).

The thermogravimetric curve 4 in Fig. 1 for cerous oxalate hemihydrate [$Ce_2(C_2O_4)_3.0{\cdot}5H_2O$] shows that there was no perceptible loss in weight till 240°C. (ab), and the rate of heating was slowed down beyond 260°C. and a temperature of 280°C. was reached during the course of about 3 hr. There was a steep fall in weight in this interval (bc) and the sample did not suffer any loss in weight between 300° and 400°C. (cd). The final product left behind was ceric oxide.

It is possible to infer from the above results that the hydrated cerous oxalate crystals lose almost all the water below 240°C. It is practically impossible to remove the last traces of water present in the crystals without decomposing the oxalate. The decomposition and the destruction of the compound takes place beyond 240°C. and these two stages cannot be distinguished.

It was felt that it may be of interest to find out whether oxygen present in air influences the decomposition of cerous oxalate. Thermogravimetric studies were, therefore, made in an atmosphere of nitrogen. Curve 5 in Fig. 1 indicates thermogravimetric behaviour of cerous oxalate hemihydrate [$Ce_2(C_2O_4)_3.0{\cdot}5H_2O$]. This is found to be similar to the one observed in air.

The present observations show that the water of hydration present in the cerous oxalate crystals is not uniformly bound to the crystals. The major portion of water (90 per cent of the water content) is more loosely held than the rest. This is very easily lost between 120° and 140°C. The lowest hydrate that can be prepared is the hemihydrate and it is practically impossible to get an anhydrous salt without damaging the oxalate. The observations of Caro and Loriers lend further support to this behaviour of

cerous oxalate. They have reported that they could locate a region in the thermogravimetric curves of cerous oxalate corresponding to $0{\cdot}5H_2O$ at about 200°C.

Further work is in progress to elucidate the nature of the water of hydration in the cerous oxalate crystals.

The authors wish to express their grateful thanks to Prof. M. R. A. Rao for his interest in the work.

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ISOLATION OF ANACARDIC ACID FROM CASHEWNUT SHELLS BY THE USE OF ANION-EXCHANGE RESIN

A method for the preparation of anacardic acid in a fairly pure form from cashewnut shells is described, in which an alcoholic extract of the shells is subjected to ion-exchange process using Amberlite IRA-400 resin.

Anacardic acid ($C_{22}H_{32}O_3$) is the chief component of the cold solvent extract of cashewnut shells. The structure of anacardic acid and the olefinic components has been extensively studied by Izzo and Dawson[1] and Paul and Yeddanapalli[2]. In the commercially available cashewnut shell liquid, the chief component is cardanol ($C_{21}H_{32}O$), the monophenolic decarboxylated product. This is due to the use of heat in splitting the raw cashewnuts to obtain the cashewnut kernels intact. To obtain anacardic acid, it is necessary to extract the shells at as low a temperature as possible to prevent decarboxylation of the acid (yielding cardanol) by using suitable solvents. Wassermann and Dawson[3] isolated anacardic acid from cashewnuts by solvent extraction and the lead salt method. The present investigation was directed towards using ion-exchange resins for the isolation of anacardic acid from the solvent extracts of cashewnuts.

Raw cashewnuts were hand broken to small pieces and the whole or broken kernels were removed from the shells. The broken shells were kept in contact with excess alcohol for a period of 48-72 hr and were periodically shaken. When the extract was deep coloured, it was filtered and the shells were again kept in contact with a fresh quantity of alcohol to extract any further remaining liquid in the shells. The filtered extract was percolated through a column of anion-exchange resin Amberlite IRA-400 in the hydroxyl form. The resin after regeneration was suspended in alcohol before percolating the alcoholic extract of the shell. The solution was percolated through the column at a slow rate and the effluent collected. After passing all the shell extract, the resin column was rinsed with alcohol to remove all physically absorbed substances and then alcoholic hydrochloric acid (5 per cent) was percolated at a very slow rate. Immediately the effluent and the liquid in contact with the resin turned deep yellow and the colour of the resin also changed. Sufficient acid was percolated to remove all the yellow solution from the column. After this the column was again backwashed and rinsed down with alcohol and the yellow acidic alcoholic solution was combined with the original acid effluent. The column was then regenerated with alkali and kept ready for another cycle of operation. In order to isolate anacardic acid, the alcoholic hydrochloric acid effluent was concentrated to a small volume by vacuum distillation and then ether extracted. The ether layer was dried over anhydrous sodium sulphate. The dried layer crystallized on keeping at 0°C. in the refrigerator. Solvents can be recovered for reuse. The refractive index of the sample was determined and the value obtained was $[n]_D^{27°} = 1{\cdot}5115$. The refractive index of pure anacardic acid as reported in literature[4] was $[n]_D^{28°} = 1{\cdot}5164$.

A yield of 600 g. of anacardic acid was obtained from 10 lb. of cashewnuts (or 4·75 lb. shells) using a 1·8 in. diam. column of Amberlite IRA-400 resin (300 g.).

The alcohol extract of cashewnut shells has been shown to contain various subsidiary constituents besides the major phenolic and acidic components[5]. While attempting to isolate the anacardic acid component only in a fairly pure condition, it is necessary to consider the adsorption and elution of the minor components also from the resin column. In the present case, by the use of alcoholic hydrochloric acid alone, it has been possible to displace the adsorbed anacardic acid from the column. Eves and Munday[6] used an anion-exchange resin in the bicarbonate form to separate organic acids from phenols. In this study also a few experiments were conducted after converting the resin to the bicarbonate form.

No substantial improvement in the yield of anacardic acid was observed and hence the resin was used only in the hydroxyl form.

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MEASUREMENT OF VISCOSITY OF LIQUIDS BY POISEUILLE'S METHOD — A CORRECTION

A new correction factor, which takes into account the average excess pressure at the exit of a tube during the time of drop formation, has been suggested to be introduced in the Poiseuille equation for pressure ($H\rho g$) in terms of the rate of flow (V). Experiments carried out with water and glycerine under low pressure heads to verify the modified equation show that the variation of H with V is linear under low rates of flow as predicted by the modified equation when the term (V^2/π^2a^4g) is negligibly small.

THE APPLICATION OF POISEUILLE'S EQUATION FOR THE determination of viscosity of liquids flowing through long, narrow tubes under a small pressure difference necessitates correction for two factors[1]. The pressure difference is partly utilized in imparting kinetic energy to the liquid, although to a negligible extent, in certain cases. The second correction is due to the acceleration not reducing to zero immediately as the liquid enters the tube. When these two corrections are applied to the Poiseuille's equation, it takes the form

$$P = \frac{8\eta(l+1{\cdot}64a)}{\pi a^4} \cdot V + \frac{V^2\rho}{\pi^2 a^4} \quad \ldots\ldots\ldots (1)$$

If P is expressed as $H\rho g$, equation (1) can be written as

$$H = \frac{8\eta(l+1{\cdot}64a)}{\pi a^4 \rho g} \cdot V + \frac{V^2}{\pi^2 a^4 g} \quad \ldots\ldots\ldots (2)$$

For determining viscosity using equation (2), several rates of flow are employed under different pressure heads and the values of $H - \frac{V^2}{\pi^2 a^4 g}$ are plotted against V. From the slope of the straight line thus obtained η is obtained.

It is observed from experiments by the original conventional Poiseuille's method where the liquid drops from the outlet end of the narrow tube, that the above straight line does not pass through the origin but has an intercept on the y-axis. Hence it is proposed to rewrite the equation as

$$H = \frac{8\eta(l+1{\cdot}64a)}{\pi a^4 g\rho} \cdot V + \frac{1}{\pi^2 a^4 g} \cdot V^2 + H_o \quad \ldots\ldots\ldots (3)$$

where H_o is the intercept on the ordinate in the above plot. The significance of H_o can be explained as follows: During the formation of the drop at the free end of the tube the pressure difference does not remain constant. When a drop just commences to form, the pressure at the exit is atmospheric. When it is about to get detached the pressure is slightly greater than the atmospheric pressure. This variation in pressure brings about the consequent variation in the rate of flow during the time of a drop formation. What is experimentally determined is the average rate of flow corresponding to an average pressure

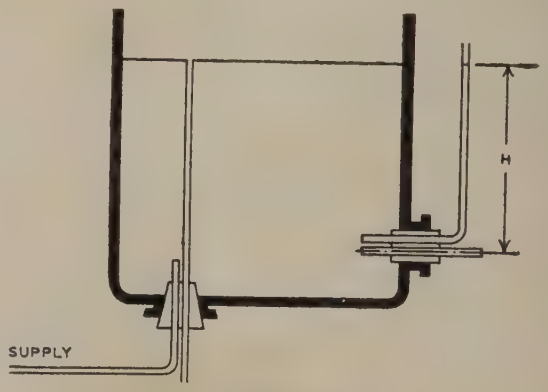

FIG. 1 — EXPERIMENTAL SET-UP

TABLE 1 — VISCOSITIES OF LIQUIDS

WATER [*Temp.*, 22°C.; l = 22·086 *cm.*; a = 0·035722 *cm.*; *and* ρ = 0·9978 *g./ml.*]		GLYCERINE [*Temp.*, 24·8°C.; l = 19·891 *cm.*; a = 0·11986 *cm.*; *and* ρ = 1·258 *g./ml.*]	
H *cm.*	$V \times 10^3$ *ml./sec.*	H *cm.*	$V \times 10^3$ *ml./sec.*
1·2555	1·8942	1·9141	1·1939
1·6595	3·2735	2·6911	1·9011
2·3175	5·0974	3·6571	2·8028
3·2355	7·8257	4·3821	3·4405
4·3165	10·9760	5·2201	4·1905
5·1395	13·4250		
Slope = 340·04		Slope = 1104·7	
η from slope = 0·0096075 poise		η from slope = 5·4997 poise	
η from data = 0·009579 poise		η from data = 5·6000 poise	
H_o intercept = 0·5842 cm.		H_o intercept = 0·5911 cm.	

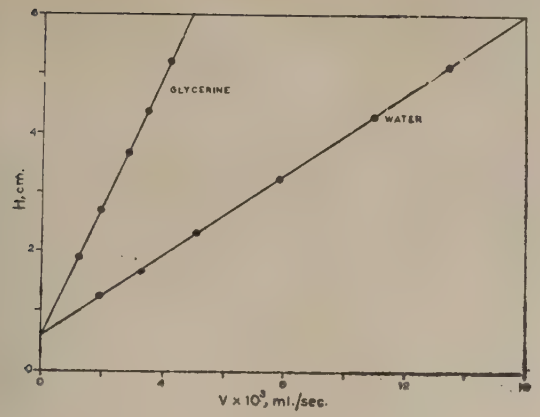

FIG. 2 — VARIATION OF H WITH V

difference. Thus H_o stands for the average excess pressure at the exit of the tube.

Experiments were conducted on water and glycerine using precise methods for determining the difference in pressure, radius and the length of the tube. The experimental set-up is shown in Fig. 1. The observations and results are given in Table 1.

The results show that the average value of H is constant for various rates of flow since the variation of H with V is a linear one (Fig. 2) and H_o is obtained from the intercept on the ordinate. It is also seen that the value of H is the same for both the liquids.

It must be pointed out that this method is a simpler one for very low pressure differences as compared to those which eliminate the drop formation at the exit end of the narrow tube by the introduction of an additional glass tube[2]. Even in those methods the fluctuation in the pressure difference exists as the drops break from the additional tube, and requires to be eliminated.

The symbols used in the equations are explained below:

a = radius of the tube, cm.
g = gravitational constant, cm./sec.^{2}
H = total head, cm.
H_o = average excess pressure, cm.
l = length of the tube, cm.
P = pressure difference, dynes/sq. cm.
V = volume rate of flow, ml./sec.
η = viscosity coefficient, poise
ρ = density, g./ml.

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VISCOMETRIC STUDIES OF CELLULOSE IN JUTE FIBRE DURING MECHANICAL PROCESSING

The effects of mechanical processing of jute and mesta fibres into yarn and reduction of fibre size in a laboratory mill have been investigated to find out whether these treatments cause any chemical degradation of cellulose. Cuprammonium viscosity (hence D.P.) determinations of α-cellulose extracted from raw fibre and batched fibre, breaker and first drawing sliver, and yarn and ground fibre show that there is no appreciable variation in the viscosity of cellulose from these sources, except in the case of cellulose from ground material, which shows definite degradation.

OPINION IS DIVIDED AS TO WHETHER OR NOT NORMAL mechanical processing of seed cotton into yarn causes any chemical degradation of the cellulose as shown by a decrease in its viscosity and consequently its degree of polymerization (D.P.). Whitwell *et al.*[1-3] have claimed that this degradation is significant, but others[4-7] have concluded that chemical degradation is effected by more drastic mechanical treatment. Though the problem is of considerable interest both to cotton and jute industries, no attempt seems to have been made to study the effect of mechanical processing of jute fibre into yarn on the D.P. of cellulose. Preliminary results of such a study on two jute fibres and one mesta fibre of different quality, selected at different stages from batching to spinning operations are reported here.

About 200 g. of material from each batch were selected, under standard conditions[8], at different stages of manufacture, viz. (1) control or raw fibre, (2) batched or softened fibre, (3) breaker sliver, (4) sliver first drawing and (5) yarn. The materials were conditioned at ordinary temperature and humidity, then cut into short lengths (3 mm.) with scissors and well mixed. About 50 g. of each were sampled out for analysis. Some were further disintegrated in a Christy and Norris laboratory mill to obtain material of 60-80 mesh. All the samples were extracted with a mixture of equal volumes of dry alcohol and benzene in a soxhlet apparatus to remove the natural fat and wax and batching oil. They were further extracted with hot water to remove the emulsifying soap used in the batching emulsion and dried in air. Replicate preparations of alpha-cellulose were made from the chlorite holocellulose of the treated material with 17·5 per cent caustic soda solution by the A.C.S. method. The samples were then opened and conditioned, and their moisture contents determined. Samples for the determination of relative viscosity, $\eta_{0\cdot5}$ (hence D.P.) at 0·5 per cent (on dry weight) concentration in cuprammonium solution were then weighed out. Intrinsic

TABLE 1 — EFFECT OF MILL PROCESSING ON ALPHA-CELLULOSE OF JUTE AND MESTA FIBRES

Stage at which sample was collected	Sample					
	6819 *C* Q 103 C 20		5847 *O* Q 65 C 28		5846 M Q 71 C 27	
	$\eta_{0\cdot5}$	D.P.	$\eta_{0\cdot5}$	D.P.	$\eta_{0\cdot5}$	D.P.
Raw fibre	6·2	928	5·4	856	7·0	993
Batched fibre	6·4	946	5·4	856	7·3	1016
Breaker sliver	6·4	946	5·8	893	7·8	1050
Sliver first drawing	6·5	954	5·7	884	7·6	1037
Yarn	6·6	962	5·7	884	7·6	1037

C, capsularis; *O*, olitorius; M, mesta; Q and C are the quality ratio and coefficient of weight variation respectively of standard yarn.

TABLE 2 — EFFECT OF GRINDING ON ALPHA-CELLULOSE OF RAW FIBRE

Sample	Cut into short lengths		Cut, ground and screened		Fall in D.P. %
	$\eta_{0\cdot5}$	D.P.	$\eta_{0\cdot5}$	D.P.	
J	6·4	946	5·6	874	7·6
J	5·2	836	4·8	794	5·0
M	7·2	1008	6·6	962	4·6
M	6·8	978	6·4	946	3·3

J, jute; M, mesta.

viscosity, as an index of the molecular weight or D.P., was obtained from the relative viscosity data. The methods of determination have been described in an earlier communication[9].

The results representing the mean values of several estimations are shown in Tables 1 and 2. It will be seen from the results in Table 1 that the mechanical processing of jute and mesta fibres does not result in decrease in the viscosity of cellulose but rather tends to increase it, indicating that cellulose undergoes no appreciable chemical degradation during mechanical processing. The increase in the viscosity may be explained as follows. The waste resulting from the original sample subjected to mechanical processing mainly consists of short and immature fibres with relatively low molecular weight (and low viscosity) cellulose, and its removal would raise the viscosity of the cellulose at successive stages of processing. The effect of grinding (Table 2) is more drastic than ordinary processing. Cellulose in the lignocellulosic fibre is apparently degraded as shown by the fall in viscosity and D.P. This is in line with the observations on pure cellulose reported by other workers[4-7,9].

Our thanks are due to Dr P. B. Sarkar, Director, for his keen interest in the work and to Dr S. B. Bandyopadhyay for physical tests.

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18 *March* 1960

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Carbohydrate Constituents of the Mycelium of *Penicillium chrysogenum* Grown in Media with Different Sources of Carbon

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Penicillium chrysogenum **has been grown in a synthetic medium with 15 different carbohydrates separately as the sole carbon source. The mycelia obtained, as well as samples from fermentors and seed tanks of the factory, have been analysed for their carbohydrate content, fractionating each into cold water-soluble sugars and residual polysaccharides. The mycelia have been found to contain 5·5–16·7 per cent of cold water-soluble free reducing sugars, glucose being the predominant sugar in all cases, accompanied by a little ribose, three disaccharides giving glucose on hydrolysis, and a compound yet to be identified. The polysaccharide content of the mycelia has been found to vary from 11·9 to 28·0 per cent and an inverse correlation has been observed between the percentage of reducing sugar in the aqueous extract and the polysaccharide content. On hydrolysis, the polysaccharides give mostly glucose in all cases.**

The seed mycelia stand out as a class by themselves in that the total carbohydrate content is low; the quantity of free reducing sugar in the aqueous extract is also low (0·8–1·6 per cent) and the sugars in this consist of fructose and ribose with a little glucose. Hydrolysis of the aqueous extract releases more reducing sugar which has been identified as galactose.

THE carbohydrate constituents of the mycelium of *Penicillium chrysogenum* grown under different conditions, have been reported by many workers. The presence of mannitol[1-3], galactose[2,4], α,α′-trehalose[2], erythritol[3], a polysaccharide containing glucose, mannose and galactose[3,4], pentosan and cellulose[5] have been reported, only qualitatively. Shu and Thorn[6] grew *P. chrysogenum* Q 176 in shake flasks on Czapek-Dox medium containing, separately, 5 per cent of glucose, galactose and xylose as the sole carbon source, and found that the mycelium contained about 60 per cent of total carbohydrates and the hydrolysates of this in all cases consisted mostly of glucose. Kita and Peterson[7] isolated from *P. chrysogenum* Q 176, grown in a synthetic medium with glucose, small quantities of mannose-1-phosphate and inositol phosphate, while Sih and Knight[8] grew *P. chrysogenum* NRRL B25 in a synthetic medium with glucose and found 0·022-0·062 per cent of the phosphates of pentose, heptose, fructose and glucose in the aqueous extracts of the mycelium.

Since the quantitative composition of the mycelium can vary with the media used and the conditions of growth, a study of the effect of different sugars and carbon sources in the medium on the carbohydrate composition of the mycelium of *P. chrysogenum* when grown in shake flasks was undertaken. Accordingly, *P. chrysogenum* (strains HA-6 and HA-9 used in production in this factory) was grown in a synthetic medium with 2 per cent of sucrose, lactose, glucose, galactose, fructose, L-sorbose, mannitol, *myo*-inositol, *myo*-inosose-2, glucuronolactone, ribose, xylose, L-arabinose, glycerol, and sodium acetate, separately, as the sole carbon source. The mycelia thus obtained, and also some samples from the fermentors and seed

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vessels of the factory grown in the complex corn steep liquor, peanut meal and lactose or sucrose media, were analysed quantitatively for their carbohydrate content and constituents, fractionating them into the cold water soluble and residual constituents. It has been found that (i) irrespective of the nature of the carbon source used, the carbohydrate composition remained the same qualitatively, (ii) the aqueous extract contained considerable amount of free reducing sugars; and (iii) glucose was the predominant sugar constituent in the aqueous extract as well as the polysaccharides. The seed mycelium differed strikingly from the rest in that the aqueous extract contained a low percentage of free reducing sugars consisting of ribose and fructose and a little glucose, and hydrolysis of this aqueous extract released galactose.

A preliminary account of this work has been published[9].

Materials and methods

Carbohydrates — D-Ribose was a gift from Hoffman-La Roche & Co., Basel, sedoheptulosan monohydrate from Dr N. K. Richtmyer of the National Institute of Health, Bethesda, Md., and 2,4-ethylidene-D-erythrose from Dr H. S. Isbell of the National Bureau of Standards, Washington, D.C. *Myo*-inosose-2 was prepared by the oxidation of *myo*-inositol with *Acetobacter suboxydans* according to the method of Posternak[10]. The other sugars were obtained from Eastman Kodak, E. Merck or British Drug Houses. All the sugars gave only one spot when paper-chromatographed; glucuronolactone, however, gave two spots, the lower, faint one corresponding to glucuronic acid.

Organism — Two strains of *P. chrysogenum*, designated HA-6 and HA-9, employed in regular production in this factory, were used. These were selections from the Russian 'new hybrid' strain derived by heterokaryosis from a white spore mutant of Q 176 and Wis. 51-20. The strain HA-3, used for production of the batch F-1374, was a white spore mutant derived from Wis. 51-20C.

Media for growth — The synthetic medium of Gitterman and Knight[11] was used, except that 2 per cent of the carbohydrate or other carbon compound being tested was added as the sole carbon source. The sugar was sterilized separately and added to the medium just before inoculation.

Growth of organism — About 200 ml. of broth from a 'seed vessel' of the factory, just before transfer to inoculate the fermentors, were collected aseptically, centrifuged, and the supernatant liquid poured off. The cells were resuspended in sterile 0·025*M* phosphate buffer (*p*H 7·0) to the original volume. Ten ml. of this suspension were used to inoculate each 500 ml. Erlenmeyer flask containing 100 ml. of the sterilized medium. The flasks were placed on a rotary shaker running at 250 r.p.m. in a room maintained at 24°C. When the sugar in the medium was exhausted, or the growth did not appear to increase, the mycelium was filtered off on a Buchner funnel, and washed by resuspension in distilled water and filtration. The mycelium was pressed well on the funnel to remove as much water as possible and then dried, first in the air and finally in a vacuum desiccator.

Seed mycelia — The seed mycelia (S-1, S-2, S-3 and S-4) were grown in the 500-gallon seed vessels of the factory, in a corn steep liquor, sucrose medium, under aeration and agitation for 48 hr after inoculation with spores. The strains used were HA-6 in the case of S-1, and HA-9 in the case of S-2, S-3 and S-4. About 500 ml. of the broth samples, before transfer to inoculate the fermentors, were collected, filtered, washed with water, pressed well and dried as before. Sample S-3 was dark and oily and was still taken up for analysis to know the degree of variation. The mycelial content in these seed broth samples was about 0·7 per cent (wt/vol.).

Fermentor mycelia — The samples of fermented broth from the regular production batches (F-1374, F-2380 and F-2382) in the factory were collected at the end of fermentation (93-114 hr) and the mycelia isolated as described before. Sample F-1374 was from a fermentor where strain HA-3 was used and the medium consisted of corn steep liquor, peanut meal and 4 per cent lactose. Samples F-2380 and F-2382 were batches where strain HA-9 was used. F-2380 was grown in corn steep liquor, peanut meal and 4 per cent lactose medium, while F-2382 was grown in a medium of corn steep liquor and peanut meal with total of 4 per cent sucrose added periodically. The mycelial content in all cases was 2·5-3·0 per cent (wt/vol.).

Fractionation of mycelia and analytical methods — About 100 mg. of the finely powdered, dry mycelium, accurately weighed, were taken in a 12×100 mm. pyrex test tube and kept with 5 ml. of ether overnight. The ether extract was removed after centrifugation and the extraction repeated twice more using 5 ml. of ether each time. The defatted mycelium was extracted thrice, as above, with isopropyl alcohol, after which it was triturated with 5 ml. of distilled water, allowed to stand overnight at room temperature (this was essential for complete extraction), centrifuged and the supernatant collected by decantation.

The residual mycelium was again extracted twice with 2·5 ml. of water, shaking and keeping for 1-2 hr at room temperature each time before centrifuging. The combined aqueous extracts were made up to 10 ml. (A), and estimated by (i) the anthrone method[12]

for total carbohydrates, (ii) the method of Somogyi[13] for reducing sugar, and (iii) the periodate method[14] to detect sugar alcohols by comparison. Glucose was used as the standard in each case.

The residual mycelium was treated with 1 ml. of 80 per cent sulphuric acid for 2-3 hr when it mostly went into solution, and then diluted with water to 10 ml. A brown gelatinous precipitate, probably chitin, separated, which was filtered off. The carbohydrate in the filtrate (B), which has been taken as the polysaccharide, was estimated by the anthrone method. Part of the acid solution (B) was heated on a boiling water bath for 3 hr to hydrolyse the polysaccharides and the reducing sugar in the resulting hydrolysate (C) was estimated by the Somogyi method.

In the case of the seed and two other mycelia, the aqueous extract (A) was also hydrolysed by adding sulphuric acid to give a 1*N* solution and heating on a boiling water bath for 30 min. The resulting hydrolysed aqueous extract (A-h) was estimated by the Somogyi or anthrone method for sugar content.

Other estimations — Total hexoses were determined by the cysteine-sulphuric acid reaction[15], ketoses by the cysteine-carbazole reaction[16], and pentoses by the orcinol reaction[17]. The nitrogen of the mycelium was determined by the Kjeldahl method taking about 100 mg. of the sample.

Paper chromatography — Paper chromatography of extracts (A) and hydrolysates (C and A-h) was carried out on Whatman No. 1 filter paper (19×40 cm.) using the descending technique with isopropyl alcohol-water (4: 1) as solvent. The sugars were located by dipping the chromatogram in dip reagents consisting of silver nitrate[18], aniline phthalate[19] (acetone was used as a solvent instead of butanol) or phloroglucinol[19] (10 ml. of glacial acetic acid, 1 ml. of hydrochloric acid, 90 ml. of acetone and 2·5 ml. of 5 per cent phloroglucinol in alcohol). Samples for paper chromatography were prepared by passing through Dowex 50 (H^+) in the case of the aqueous extracts, or neutralizing with barium carbonate and then passing through Dowex 50 (H^+) in the case of the hydrolysates (C and A-h). The resulting solutions were evaporated to dryness at room temperature in a vacuum desiccator, dissolved in 1-3 drops of distilled water and then applied to paper.

Identification of spots — For identification of spots, the areas corresponding to the spots in the paper chromatograms were cut out and the sugar eluted by the method of Flood, Hirst and Jones[20]. About 20-50 μg. were taken for the presulphonated and unsulphonated resorcinol reaction spectra. A sugar-free portion of filter paper of same area was cut out from the chromatogram, eluted with water, and the eluate served as the blank.

Unsulphonated and presulphonated resorcinol spectra — To identify the sugar present in solutions, the presulphonated and unsulphonated resorcinol reaction spectra were taken according to the method of Devor *et al.*[21]. About 50 μg. of the sample were used, and the optical densities measured in the region of 360-560 mμ, using a Beckman spectrophotometer, model DU. Standard spectra were taken with glucose, galactose, mannose, rhamnose, fructose, sorbose, xylose, arabinose, ribose, erythrose, sedoheptulose, glucuronolactone, inosose-2, dihydroxyacetone, sucrose, lactose and trehalose. Mannitol and inositol did not give any colour. The absorption spectra of some of the sugars, besides glucose, galactose, mannose, xylose and arabinose already reported[21], are given in Fig. 1. It is evident from Figs. 1 and 2 that each sugar has a characteristic spectrum of its own in the unsulphonated resorcinol reaction and by integrating this with the spectrum obtained with presulphonated resorcinol reagent, it is possible to identify the individual sugars. The spectra of the disaccharides show resemblance to the component sugars. This method of identification of sugars is extremely useful in the work on sugar metabolism and effectively supplements the orcinol, cysteine-sulphuric acid and cysteine-carbazole reactions.

Results

Growth of mycelia — Table 1 gives the results obtained in a typical growth experiment for each carbon source tried. Apparently fastest growths were observed with glycerol, ribose and sorbose which were used up in about two days. Growth was poorest with sodium acetate, inosose-2 and glucuronolactone; the last two were present in the medium to the extent of 0·3 and 0·5 per cent (wt/vol.) at the end of the growth period, and in these cases, the dry weight of the mycelia were also lowest.

While growth with inosose was slow, that with inositol was indeed good; this indicates that the metabolism of inositol leading to glucose synthesis does not proceed through the oxidation product inosose-2[22] but follows a different course[23]. Lactose and mannitol gave slow rates of growth. The mycelial dry weight varied between 0·83 and 0·28 g./100 ml. of the medium, the maximum being given by sucrose, glucose and mannitol and the minimum with sodium acetate, inosose-2, glucuronolactone and inositol.

The nitrogen content of the mycelia (Table 2) varied between 4·79 and 7·10 per cent, being highest with sorbose- and glycerol-grown mycelia, and lowest with mannitol-, sodium acetate-, glucuronolactone-

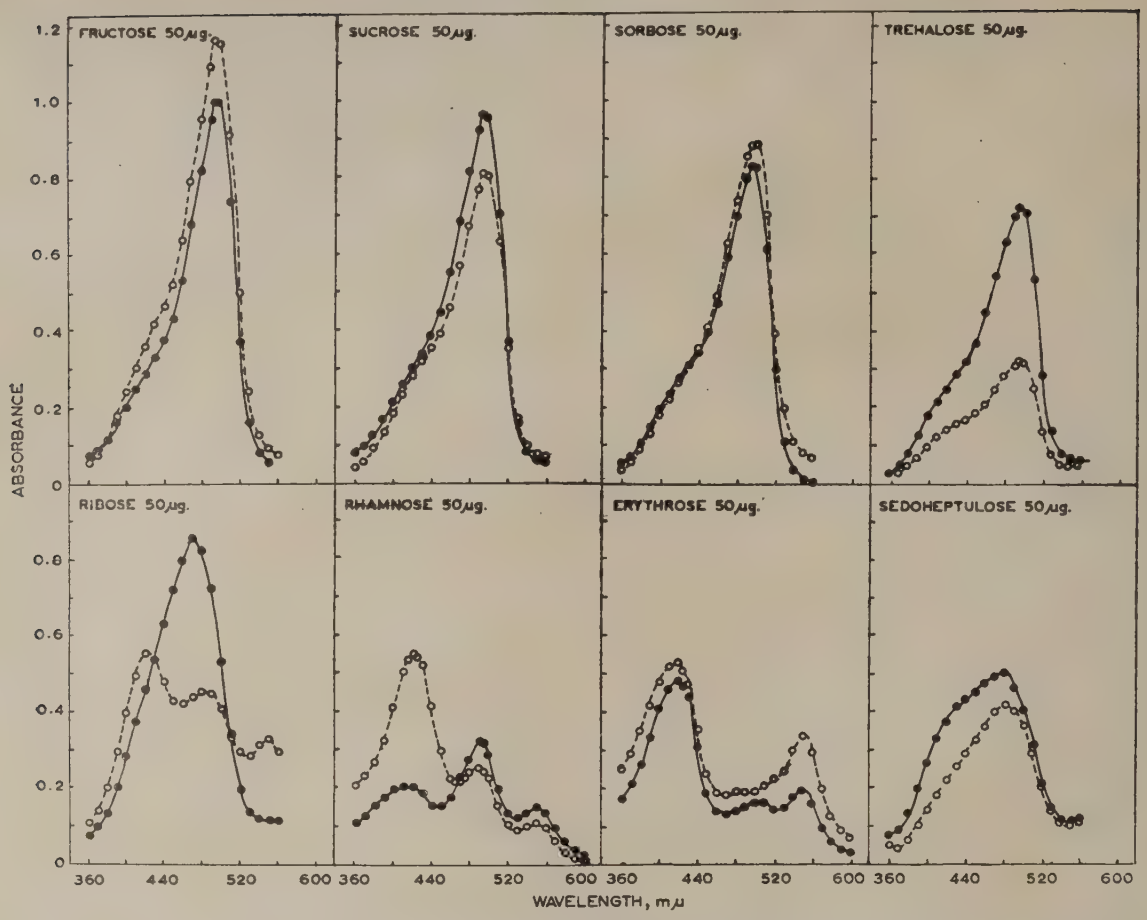

FIG. 1 — ABSORPTION SPECTRA OF VARIOUS SUGARS IN THE RESORCINOL-SULPHURIC ACID REACTIONS [o - - - o, unsulphonated resorcinol reaction; • —— •, presulphonated resorcinol reaction]

TABLE 1 — GROWTH OF *P. CHRYSOGENUM* ON SYNTHETIC MEDIUM WITH VARIOUS CARBON SOURCES

CARBON SOURCE	STRAIN USED	NATURE AND DEGREE OF GROWTH	GROWTH PERIOD *hr*	FILTRATE		MYCELIUM (*g./100 ml. broth*) %
				*p*H	Sugar %	
Sucrose	HA-6	Good, pellety	72	6·5	—	0·83
Lactose	HA-6	Slow	97	6·0	—	0·57
Glucose	HA-6	Good, pellety	72	6·5	0·08	0·81
Galactose	HA-6	Good	72	6·5	0·10	0·70
Fructose	HA-6	do	72	7·0	0·03	0·57
Sorbose	HA-6	Very good	51	6·0	0·19	0·70
Mannitol	HA-9	Slow, pellety	187	5·5	—	0·81
myo-Inositol	HA-6	Good	73	6·0	0·00	0·45
myo-Inosose-2	HA-9	Slow, pellety	187	5·6	0·30	0·39
Glucuronolactone	HA-9	do	187	6·1	0·50	0·42
Ribose	HA-6	Very good	50	6·5	0·03	0·73
Xylose	HA-6	Thick, pellety	72	6·8	0·02	0·57
L-Arabinose	HA-6	Good	71	6·0	0·01	0·67
Glycerol	HA-6	Very good	48	4·0	—	—
Sodium acetate	HA-6	Poor	120	9·5	—	0·28

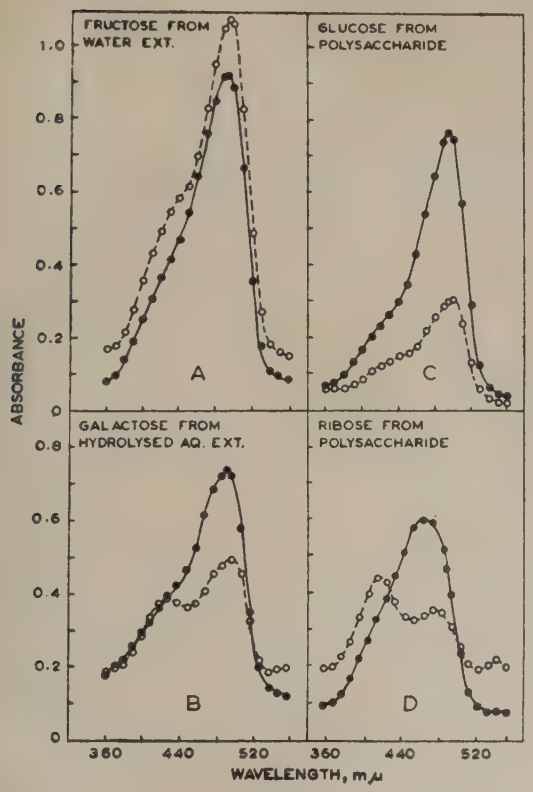

FIG. 2 — ABSORPTION SPECTRA OF SUGARS ELUTED FROM PAPER CHROMATOGRAMS OF VARIOUS EXTRACTS OF SEED MYCELIUM OF *P. chrysogenum* IN THE UNSULPHONATED RESORCINOL (o - - - o) AND PRESULPHONATED RESORCINOL (• —— •) REACTIONS

and inositol-grown mycelia. There was little variation in the other cases.

No correlation was observed between the rate of growth on the one hand and the dry weight, nitrogen content, the sugar percentage in the aqueous extract or the total polysaccharide on the other.

Carbohydrate content of mycelia

Since the seed mycelia stood out as a class by themselves, they are treated separately.

Isopropyl alcohol extract — Paper chromatography carried out with the isopropyl alcohol extracts of five mycelia revealed the presence of mannitol; another spot with R_G 1·26 also revealed by silver nitrate dip reagent is probably another sugar alcohol (erythritol ?).

Cold water extracts (*A*) — In one case, the mycelium was extracted at room temperature with water for 1, 2, 4 and 16 hr. The quantity of sugar extracted increased progressively, the hexose as a percentage of the total carbohydrate was about the same in all extracts, and the paper chromatography of each extract revealed the same distribution of the sugars. The 16 hr as well as the overnight extraction gave agreeing values for sugar content. So, overnight extraction was adopted in all cases as a standard procedure.

In Table 2 are given the sugar contents of the aqueous extracts of the mycelia as determined by the anthrone, Somogyi and periodate methods. The results by the three methods agree fairly well in all

TABLE 2 — CARBOHYDRATE COMPOSITION OF THE MYCELIUM OF *P. CHRYSOGENUM*

Carbon source	Water-soluble sugar %			Polysaccharide %		Total carbohydrate %		Aq. extract as % of total $\left(\frac{A}{A+B}\times 100\right)$	N %
	Anthrone (A)	Somogyi	Periodate	Anthrone (B)	Somogyi	Anthrone (A+B)	Somogyi		
Sucrose	14·2	17·5	19·0	11·9	13·2	26·1	30·7	54·4	5·75
Lactose	11·0	12·8	13·1	14·2	16·2	25·2	29·0	43·6	6·13
Glucose	7·0	7·6	6·8	26·4	30·6	33·4	38·2	26·9	5·86
Galactose	10·5	12·0	9·6	19·7	21·4	30·2	33·4	34·7	6·20
Fructose	14·5	15·8	16·8	15·8	17·6	30·3	33·4	47·7	5·65
Sorbose	6·9	7·8	6·1	21·3	25·9	28·2	33·7	24·4	7·01
Mannitol	13·0	13·0	15·3	23·4	24·6	36·4	37·6	35·7	4·79
myo-Inositol	10·4	10·6	11·8	21·3	23·5	31·7	34·1	32·8	5·09
myo-Inosose-2	9·8	10·2	12·3	23·8	25·2	33·6	35·4	29·1	5·24
Glucuronolactone	12·6	12·9	16·3	17·4	17·4	30·0	30·3	40·2	5·05
D-Ribose	13·8	15·4	15·5	21·0	24·3	34·8	39·7	39·6	6·09
D-Xylose	12·7	13·9	13·7	18·9	21·6	31·6	35·3	40·2	6·06
L-Arabinose	16·7	19·2	20·7	14·9	17·7	31·6	36·9	52·8	6·17
Glycerol	9·2	11·0	10·2	15·8	19·4	25·0	30·4	36·8	5·75
Sodium acetate	5·5	5·9	5·1	15·1	19·7	20·6	25·6	26·7	4·83
F-1374	6·8	6·4	12·0	28·0	34·0	34·8	40·4	19·5	3·65
F-2380	12·4	13·3	14·3	19·4	23·8	31·8	37·1	39·0	4·25
F-2382	8·9	9·7	12·6	20·6	25·8	29·5	35·5	30·1	4·49
S-1	0·7	0·8	1·2	12·2	14·4	12·9	15·2	5·4	4·73
S-2	1·9	2·7	1·7	10·8	12·0	12·7	14·7	14·9	5·24
S-3	0·8	1·0	1·0	6·2	7·6	7·0	8·6	11·4	2·84

The carbohydrate percentages were calculated on dry weight of mycelium with reference to glucose as the standard. At least two samples were assayed in each case and the mean of agreeing duplicate values are given in the table.

cases except one, indicating that the aqueous extracts contain almost entirely free reducing sugars, which vary from 5·5 to 16·7 per cent of the weight of the mycelium. The acetate-grown mycelium gave the lowest figure, with glucose-, sorbose-grown and F-1374 mycelia coming next. The arabinose-grown mycelium contained the highest percentage, with fructose- and sucrose-grown ones coming close to it. The striking fact is that the sugars in the aqueous extracts constitute from 19·5 to 54·4 per cent of the total carbohydrates of the mycelia, the sorbose-, sodium acetate- and glucose-grown mycelia giving 24·4, 26·7 and 26·9 per cent of the total, and sucrose-, arabinose- and fructose-grown mycelia 54·4, 52·8 and 47·7 per cent of the total respectively.

Polysaccharides — The polysaccharide contents of the mycelia (excluding chitin) are given in Table 2. The results by the two methods agree fairly well, which indicates that the polysaccharide is easily hydrolysed. The polysaccharide content (anthrone estimation) varies from 11·9 to 28·0 per cent, the lowest being in the surcose- and lactose-grown mycelia and the highest in F-1374 and glucose-grown mycelia. There appears broadly to be an inverse correlation between the percentages of cold water-extracted sugar and the polysaccharide content[9].

Total carbohydrate — The total carbohydrate content (aqueous extract plus polysaccharide) of the mycelia (Table 2) varies from 20·6 to 36·4 per cent. Mannitol-, ribose-, *myo*-inosose-2-, glucose-grown mycelia and F-1374 give the highest figures and the acetate-, glycerol-, lactose- and sucrose-grown mycelia the lowest. The variation in the total carbohydrate, barring the two extremes, is not so much as with the content of polysaccharide or the cold water-extracted sugar. There seems to be no correlation between the total carbohydrate content and the rate of growth, the dry weight of mycelium or percentage of nitrogen. The values for carbohydrate content obtained here are much lower than those reported by Shu and Thorn[6], who used, however, 5 per cent of the carbohydrates as the carbon source.

Seed mycelia

The striking fact noticed here is that the seed mycelia, which multiply fast and do not produce penicillin[24,25], stand out as a class by themselves, sharply differing from the others. As seen from Table 2, the total carbohydrate content was low (7·0-12·9 per cent) and the aqueous extract contained only 0·7-1·9 per cent of free reducing sugars which were also qualitatively different. The sugar in the aqueous extracts (expressed as percentage of the total carbohydrates) was also low (5·4-14·9 per cent). Another interesting fact is that after hydrolysis of the aqueous extract with *N* sulphuric acid for 30 min., the percentage of free reducing sugars increased considerably, i.e. from 0·17 to 0·47 mg./ml., 1·32 to 1·92 mg./ml. and 0·50 to 0·98 mg./ml. in the case of S-1, S-2 and S-3 respectively. The other two mycelia (glucose-grown and F-2380) taken for comparison do not show this high increase in sugar content on hydrolysis. As will be shown later, the sugar released in the case of seed mycelia was galactose.

Identification of the sugar components

Aqueous extracts (*A*) — All the aqueous extracts, after passing through Dowex 50 (H^+) were estimated for their sugar content, aliquots containing approximately 50 μg. of the sugar were taken, treated with the unsulphonated and presulphonated resorcinol reagents, and the optical densities taken in the region 360-560 mμ. The spectra of all the aqueous extracts, except those of the seed mycelia, were broadly the same and indicated glucose to be the predominant sugar. Hexose present in the extracts constituted 80-90 per cent of sugar in the aqueous extract and nearly 100 per cent in the hydrolysates. The absorption curves for the cysteine-sulphuric acid reaction in the region 360-530 mμ were typical of glucose. Spectra taken after 18 hr showed the absence of heptose. The blue colour, which indicates the presence of galactose or sorbose, was not observed after keeping the reaction mixture for 48 hr.

Aqueous extract of seed mycelia — The unsulphonated and presulphonated resorcinol reaction spectra of the aqueous extracts of the seed mycelia showed the presence of pentoses and also fructose. Fructose, estimated in the case of S-1, was found to constitute 25 per cent of the total carbohydrates. From the absorption curve taken in the region 450-750 mμ, there was no evidence of the presence of trioses. About 30 per cent of the total sugar in the aqueous extract of S-1 were pentose.

Paper chromatography — The sugars present in the extracts were identified by paper chromatography. Since glucose appeared to constitute a high percentage, to identify the other sugars present in small quantities in the chromatogram, sufficient quantity had to be applied which resulted in the glucose spot becoming large. It was found that the extracts of all mycelia, except seed mycelia, gave the same distribution of the sugars in the chromatograms. In Table 3 are given the results of paper chromatographic analysis of the aqueous extracts (A) of all the mycelia. While glucose is the predominant sugar, ribose (R_G 1·20) is present in all the extracts. There were four other spots of approximate R_G 0·50, 0·65, 0·75 and 1·40. To definitely identify these, the areas

corresponding to the spots on marker strips were cut out, the sugars eluted with water, and the resorcinol reaction spectra taken. The three sugars corresponding to R_G 0·50, 0·65 and 0·75 gave spectra resembling glucose. They are probably disaccharides containing glucose. One of them with R_G 0·75 may be trehalose. The spot with R_G 1·40 has yet to be identified.

As far as the seed mycelia are concerned, ribose and fructose have been detected as the predominant sugars with small quantities of glucose (Fig. 2A).

The results obtained by chromatography with the hydrolysed aqueous extract of the seed mycelia and the other mycelia are given in Table 4. It is seen that galactose comes out as the predominant sugar present and its identity has been confirmed as shown in Fig. 2B. In the case of the other mycelia, F-2380 and glucose-grown one, the pattern is different, and the presence of galactose is doubtful.

Hydrolysates (*C*) — The resorcinol spectra of the hydrolysates (C) of the mycelia, including the seed, showed that glucose is the predominant sugar.

TABLE 3 — SUGARS DETECTED BY PAPER CHROMATOGRAPHY OF THE COLD WATER EXTRACTS OF MYCELIA

Carbon source	R_G 1·00 glucose	1·20 ribose	1·40	0·50	0·65	0·75	Additional spots
Sucrose	++++	++	+	Faint	+	Faint	—
Lactose	++++	++	+	—	—	—	—
Glucose	++++	+	Faint	—	—	+	—
Galactose	++++	++	+	—	—	—	—
Fructose	++++	++	+	+	—	—	—
Sorbose	+++	+	Faint	—	—	Faint	—
Mannitol	++++	+	+	—	—	—	—
myo-Inositol	++++	+	Faint	—	+	—	—
myo-Inosose-2	++++	+	+	—	—	—	R_G 0·40++ (inosose)
Glucuronolactone	++++	+	+	—	—	—	—
Ribose	++++	+	+	—	—	—	—
Xylose	++++	+	Faint	—	—	—	—
L-Arabinose	++++	+	+	—	—	—	—
Glycerol	++++	Faint	+	Faint	Faint	—	—
Sodium acetate	+++	++	+	—	—	Faint	—
F-1374	++++	Faint	++	—	—	—	—
F-2380	++++	++	—	Faint	—	—	—
F-2382	++++	++	—	do	—	—	—
S-1	+	++	+	—	—	++	R_G 1·08+ (fructose)
S-2	+	+	—	—	—	—	R_G 1·10+ (fructose)
S-3	—	+	—	—	—	—	—
S-4	Faint	+	—	—	—	—	R_G 1·09+ (fructose)

The chromatograms were dipped in silver nitrate reagent. When dipped in aniline phthalate reagent, at R_G 1·20 pink, at R_G 1·00 olive-brown, and at R_G 1·40 faint yellow spots were obtained. With phloroglucinol reagent an orange spot appeared at R_G 1·10 for the seed mycelium. ++++ denotes a large, dark spot; +, just distinctly visible; and ++ and +++, increasingly darker spots than +.

TABLE 4 — SUGARS DETECTED BY PAPER CHROMATOGRAPHY OF THE HYDROLYSED AQUEOUS EXTRACTS

Mycelium	Dip reagent	R_G 0·90 galactose	1·00 glucose	1·10 fructose	1·20 ribose	1·40	0·45	0·30
S-1	Silver nitrate	+++	—	++	++	+	+	+
S-1	Aniline phthalate	Olive-brown	—	—	Pink	—	—	—
S-1	Phloroglucinol	—	—	Orange	—	—	—	—
S-2	Silver nitrate	++	+	+	++	+	+	Faint
S-2	Aniline phthalate	Olive-brown	—	—	Pink	—	—	—
S-4	Silver nitrate	+++	—	+	++	—	+	+
F-2380	do	?	++++	—	++	—	Faint	—
Glucose-grown	do	?	++++	—	++	—	do	—

++++ denotes a very dark, large spot; +, just distinctly visible; and ++ and +++, increasingly darker spots than +.

TABLE 5 — SUGARS DETECTED BY PAPER CHROMATOGRAPHY OF THE HYDROLYSATES OF MYCELIA

Carbon source	R_G 1·00 glucose	R_G 1·40	R_G 1·10	R_G 0·55	R_G 0·75	Additional spots
Sucrose	++++	+	+	+	—	—
Lactose	++++	+	+	+	—	—
Glucose	++++	Faint	—	Faint	+	—
Galactose	++++	+	+	+	—	—
Fructose	++++	+	+	+	—	—
Sorbose	++++	+	+	+	—	—
Mannitol	++++	+	+	+	—	R_G 0·35+
myo-Inositol	++++	—	—	++	+	—
myo-Inosose-2	++++	+	+	+	—	—
Glucuronolactone	++++	+	+	+	—	—
Ribose	++++	—	—	—	—	R_G 0·28 faint
Xylose	++++	++	+	+	Faint	—
L-Arabinose	++++	Faint	—	++	—	—
Glycerol	++++	—	—	—	—	—
Sodium acetate	++++	++	+	++	—	—
F-1374	++++	+	+	—	—	—
F-2380	++++	—	+	+	+	—
F-2382	++++	—	+	+	Faint	—
S-1	++++	+	+	++	—	R_G 1·20++ (ribose)
S-2	++	—	—	—	—	—
S-3	++	—	—	—	—	—

Chromatograms were dipped in silver nitrate reagent. With aniline phthalate reagent spots at R_G 1·00 were olive-brown, R_G 1·40 faint yellow, R_G 0·55 faint brown and R_G 1·20 pink. ++++ denotes a dark, large spot; +, just distinctly visible and ++ and +++, increasingly darker spots than +.

Estimation of the hexose content confirmed this. The hydrolysates were paper chromatographed and the results obtained are given in Table 5. In addition to the presence of glucose, four more spots were present. Ribose was absent. The spot with R_G 0·55 was not inositol but like the other of R_G 0·75 was found to be a disaccharide which had escaped hydrolysis. The spot at R_G 1·10, in the position of fructose, appeared very slowly after dipping in the silver nitrate reagent and was not revealed by aniline phthalate or phloroglucinol. Resorcinol spectrum, with the eluate of the area corresponding to the spot, showed high absorption in the ultraviolet, and low absorption in the region 400-500 mμ. This, found in all the hydrolysates, may not be a carbohydrate at all. A sufficient quantity is being collected for identification.

The resorcinol spectra of the eluates of the areas corresponding to ribose and glucose in the chromatograms of the hydrolysates of the seed mycelia are given in Figs. 2C and 2D, which definitely establish their identity.

Discussion

Penicillium chrysogenum is able to grow on a variety of carbohydrates and carbon compounds as the sole carbon source, though the rate and extent of growth is not the same in all cases. In addition, the pattern of distribution of sugars in the cellular constituents in all cases is the same, though there are quantitative variations. The mycelia grown in shake flasks in synthetic media with various carbon sources, and those in the fermentors in complex media, do not show much difference as regards the cellular constituents, though the growth in the latter case is very heavy. In general, the capacity to synthesize specific mycelial constituents is dictated by the genetic make up of the strain, while the media constituents as well as the conditions of growth affect this pattern only quantitatively. The seed mycelia are characterized by very fast rates of growth and inability to produce penicillin[24,25] and it is not surprising that they differ in some respects from the other mycelia harvested when the growth had stopped.

The rate and degree of growth of a microorganism in a medium, resulting in the synthesis of cellular constituents, are complicated processes involving many parameters about which we have little specific information. We are, therefore, not able to find direct correlations between the structures of the carbon sources used in the medium and other factors analysed. For example, from a knowledge that glucose is the predominant sugar constituent of the mycelium and is also utilized by the mould, we expect it *prima facie* to give the fastest growth; but we find that glycerol, ribose and sorbose are better in this respect.

Significant amounts of free reducing sugars, accounting in some cases to as much as 50 per cent of the total carbohydrates, are present in the cold water

extracts, which we propose to call the 'free sugar pool'. This consists mostly of glucose, with small quantities of ribose always accompanying it. As discussed below, the picture is different with the seed mycelia.

The primary step in carbohydrate metabolism concerned with mycelial growth seems to consist of the conversion of the various sugars and other carbon sources of the medium into glucose by the appropriate metabolic pathways, which would differ for the various sugars as regards their nature and rate. Glucose thus produced would feed the 'free sugar pool' and would be expended further in two ways. Firstly, it would be converted into the cellular polysaccharide, glucosan, and also chitin through conversion into glucosamine. Secondly, it would be metabolized by various pathways not only to yield energy but also provide the carbon skeleton of the amino acids to be converted into proteins, and also other cellular constituents. The size of the sugar pool in the mycelium would be decided by the rate at which the sugar is synthesized in the primary reaction to feed the pool and the rate at which it is expended in subsequent reactions as indicated above. When the mycelium is multiplying fast, there would be a heavy withdrawal from the sugar pool which consequently would get depleted. This seems to be the case with the seed mycelia, the aqueous extract of which contains little sugar and even this consists of fructose and ribose with little glucose. On the other hand, in the mycelia where the growth rate is reduced considerably, there would not be a heavy demand of glucose in the pool to synthesize the cellular constituents and so the pool would contain considerable quantities of the sugar synthesized in the primary reaction. Though we have harvested all the mycelia when the growth had ceased, there is still a variation in the quantity of sugars in the aqueous extracts and the polysaccharide content.

The glucose present in the aqueous extracts of all the mycelia we postulate to be the end product of the primary reaction feeding the free sugar pool. We do not rule out the possibility that it could also arise under certain conditions, particularly starvation, by the breakdown of the polysaccharides. It does not appear that the sugar isolated is existing as the phosphate because such large quantities would inhibit further metabolization[26]. There may be phosphatases present at the site which would convert the phosphates of the sugars into the free sugars isolated.

The presence of free ribose in the aqueous extract is of interest. In living systems, ribose seldom exists free but only as the nucleotides and nucleic acids. The ribose produced intracellularly by the hexosemonophosphate pathway would have two courses open to it — conversion into the nucleotides and recycling to glucose by the pentose pathway. What is detected in the aqueous extracts is possibly the excess left over after these two conversions. In the metabolic reaction, ribose would be functioning as the phosphate; there might be phosphatases present converting it into free ribose.

The hexosemonophosphate pathway has been found to operate in *P. chrysogenum* and it is supposed to play a major role in the earlier phases where rapid growth takes place[8,27-29]. This is conceivable because many of the vital constituents are to be supplied by this pathway. Fructose and ribose have been detected in the aqueous extracts of the seed mycelia, and ribose in the aqueous extracts of all mycelia. The other intermediates in this pathway have not been detected, and may be existing in quantities too small for detection.

The aqueous extracts of seed mycelia differ from all others in three respects: they contain only small quantities of free reducing sugars, these are fructose and ribose with little glucose, and hydrolysis of the extract apparently causes an increase of free reducing sugar which is due to galactose. In the unhydrolysed extract galactose must be existing as a complex, probably as a water-soluble galactan or a uridine derivative.

Since the mould is able to convert a variety of sugars and other carbon sources into glucose[30], it is a formidable storehouse of different enzymes involved in the various types of conversions. It thus gives us an opportunity to study the pathways of conversion of these sugars into glucose. The various sugars in our study have been chosen from this point of view. Studies along these lines both in growth experiments as well as with cell extracts are being carried out.

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Effect of Carbohydrates & Some Carbon Sources on the Biosynthesis of Benzylpenicillin by Washed Cells of *Penicillium chrysogenum*

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A variety of carbohydrates, such as glucose, galactose, mannitol, sorbitol, dulcitol, *myo*-inositol, *myo*-inosose-2, glucuronolactone, ribose, xylose, dihydroxyacetone and glycerol, have been found to stimulate the rate of biosynthesis of benzylpenicillin by washed cells of *Penicillium chrysogenum* suspended in phosphate buffer containing 0·05 per cent phenylacetate (PA) and aerated in a shaker. The rate of utilization of these sugars and their effects on mycelial weights have been determined in each case. Lactate, pyruvate, citrate, succinate and DL-malate do not show any stimulatory effect. 2,4-Dinitrophenol, cyanide and arsenite inhibit the biosynthesis of benzylpenicillin in the PA plus glycerol and PA plus inositol systems when added in concentrations of 0·0006*M*, 0·005*M* and 0·005*M* respectively. It is suggested that all the carbohydrates tried get converted into glucose and feed the water-soluble, reducing 'sugar pool' of the mycelium containing mostly glucose. The role of glucose in this pool is the continued generation of ATP molecules postulated to be essential for the biosynthesis from the immediate precursors, and the dehydrogenation also required is coupled with the flavoproteins or cytochromes, most probably the latter.

CARBOHYDRATE constitutes one of the major constituents of the media employed for penicillin production by *Penicillium chrysogenum*. A considerable portion of it is used up for the synthesis of the mycelial constituents and for the production of energy for driving the various synthetic steps connected with this. It is known that for continued production of penicillin by the mycelium, an optimum supply of carbohydrate in the medium is necessary. In order to understand the role of the carbohydrates in penicillin biosynthesis, experiments were carried out employing a system which consisted of a suspension of washed cells of *P. chrysogenum*, collected from a fermentor run at the penicillin-producing phase, in phosphate buffer containing phenylacetate (PA), and

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aerated by agitation on a rotary shaker. The compounds studied were added to the suspending medium and the stimulatory effect on penicillin synthesis over the control system was studied. By this technique, it was observed that a number of carbohydrates, fatty oils and fatty acids cause the stimulation of penicillin synthesis[1,2]. In continuation of this work, the effects of a variety of carbohydrates and other carbon sources on penicillin biosynthesis have been studied in greater detail and the results obtained are presented here. A working hypothesis has also been suggested which seems to account for all the facts so far collected.

A preliminary account of the results obtained has been published[3,4].

Materials and methods

Carbohydrates and carbon sources — D-Ribose was a gift from Hoffman-La Roche & Co., Basel. Glucose, *myo*-inositol, dulcitol and DL-malic acid were obtained from Eastman Organic Chemicals, galactose and glucuronolactone from E. Merck, and D-xylose, glycerol and sorbitol from British Drug Houses. Sodium pyruvate and sodium lactate were prepared from the respective acids by the method of Robertson[5]. *myo*-Inosose-2 was prepared by the action of *Acetobacter suboxydans* (I.C.T.C. No. H621) on *myo*-inositol according to Posternak[6] and dihydroxyacetone (DHA) similarly from glycerol as described by Underkoffler and Fulmer[7].

Organism — *Penicillium chrysogenum*, strains HA-6 and HA-9, local isolate from the Russian 'new hybrid' strain, were used.

Mycelial suspensions — Samples of fermented broth were collected from the big fermentors in the regular production runs in the factory between 35 and 47 hr when the rate of penicillin production was high. The mycelial suspensions were prepared and the experiments conducted as described by Deshpande and Ganapathi[1,2].

Assay of penicillin concentration — This was done according to the method of Humphrey and Lightbown[8]. In interpreting the results, significance is attached if the values differ by over 10 per cent. The 12-14 hr samples could not be assayed immediately and were preserved at −20°C. overnight; in some experiments a slight drop in penicillin titres was found.

Estimation of carbohydrates — Reducing sugar was estimated by the method of Somogyi[9], inositol by the periodate oxidation method[10] and glycerol and mannitol as described by Ryley[11]. Glucuronolactone was determined, after deproteinization of the solution with 40 per cent (wt/vol.) trichloracetic acid, by the method of Mejbaum as modified by Charalampous and Lyras[12], and pyruvate by the method of Friedemann and Haugen[13].

Mycelial weight — The dry weight of mycelium in the samples was determined by filtering a measured volume of the suspension on a Buchner funnel, washing with a small quantity of water, drying the mycelium first in air and finally in a vacuum desiccator and weighing.

Nitrogen content — The nitrogen content of the dry mycelium was determined by the Kjeldahl method taking about 100 mg. of sample.

Results

Each experiment was repeated two or three times to make sure of the consistency of the pattern of the results obtained. There were variations from batch to batch as regards mycelial weight and rate of production of penicillin. So a control flask with only PA was included in every experiment, and results were evaluated with reference to this. The results of typical experiments are given in Tables 1-5 and Figs. 1-6.

Effect of glycerol — The effect of adding glycerol in 0·2, 0·5 and 1·0 per cent (wt/vol.) concentrations is given in Fig. 1. At 0·2 per cent concentration, all the glycerol disappeared from the medium within 11 hr and the stimulation of penicillin synthesis over the control system was only slight. The stimulatory effects are about the same when glycerol is added in 0·5 and 1·0 per cent concentrations.

The initial suspension had a mycelial content of 1·77 per cent (wt/vol.), which fell at the end of 24 hr to 1·32 per cent in the control system without sugar and 1·49 per cent in the case of 0·2 per cent glycerol addition, probably due to autolysis of the mycelium. When glycerol was added in 0·5 and 1·0 per cent concentrations, the final mycelial weights were 1·86 and 1·77 per cent respectively.

Effect of 2,4-dinitrophenol (DNP), cyanide and arsenite on the glycerol plus PA system — When

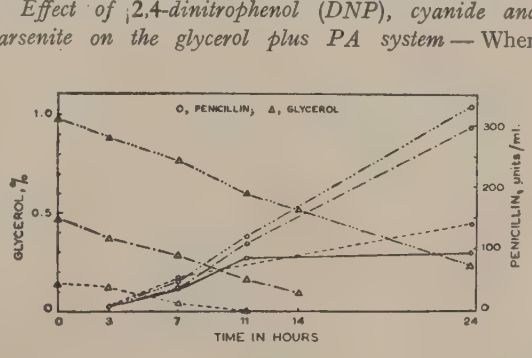

FIG. 1 — EFFECT OF GLYCEROL ON THE BIOSYNTHESIS OF BENZYLPENICILLIN [Washed cells of *P. chrysogenum* (batch No. 2073, 35 hr) were resuspended in 0·025*M* phosphate buffer (*p*H 7) with 0·05 per cent PA. ——, no carbohydrate; - - - - -, 0·2 per cent glycerol; — · —, 0·5 per cent glycerol; — · · · —, 1·0 per cent glycerol]

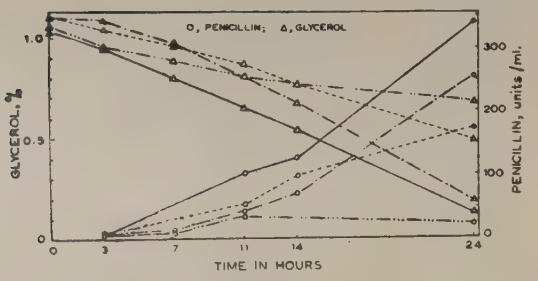

FIG. 2 — EFFECT OF INHIBITORS ON THE BIOSYNTHESIS OF PENICILLIN [Washed cells (batch No. 2071, 35 hr) suspended in 0·025*M* phosphate buffer (*p*H 7) with PA (0·05 per cent) and glycerol (1·0 per cent). Initial mycelial dry weight was 2·07 per cent and nitrogen content of mycelium 5·95 per cent. ——, no inhibitor; - - - - -, DNP (0·0006*M*); — · —, KCN (0·005*M*); — · · —, arsenite (0·005*M*)]

DNP, cyanide and arsenite were added separately in concentrations of 0·00006*M*, 0·0001*M* and 0·0002*M* to the PA plus glycerol system, there was no inhibition of penicillin synthesis. The rate of disappearance of glycerol as well as the mycelial percentage at the end of 24 hr were about the same in all cases. But when they were added in concentrations of 0·0006*M*, 0·005*M* and 0·005*M* respectively, there was inhibition of penicillin biosynthesis as shown in Fig. 2. There was almost total inhibition in the case of arsenite; it was found that pyruvate accumulated in the medium (Table 1) and the effect of arsenite was not reversed by addition of 0·5 per cent (wt/vol.) acetate as was observed in the case of erythromycin biosynthesis[14]. In the case of cyanide, there was inhibition for about 14 hr, after which there was penicillin production at a good rate; the cyanide probably gets destroyed by then. This also indicates that cyanide does not permanently damage the system involved in penicillin biosynthesis. The weights and nitrogen contents of mycelia (initial weight, 2·07 per cent and initial nitrogen content, 5·95 per cent) at the end of the experiment (24 hr) without inhibitor and with DNP, KCN and arsenate were: weight of mycelia, 2·38, 1·84, 2·03 and 1·45 per cent and nitrogen content of mycelia, 5·34, 5·54, 5·33 and 5·63 per cent respectively. In the case of DNP, there was about 50 per cent inhibition. As regards mycelial weight at the end of the experiment (24 hr), the highest decrease was observed with arsenite and less with DNP, while in the case of cyanide there was not much change from the control. The nitrogen contents of the mycelia at the end of the experiment show reverse correlation with penicillin synthesis (Fig. 2).

Effect of glycerol as compared with glucose and galactose — The results given in Table 2 show that the effect of glycerol, glucose and galactose in stimulating penicillin production is about the same. The mycelial weights show that there is the usual decrease in the control flask, while in the others there is a slight increase. The rate of disappearance of glucose and galactose is about the same, both being used up within 14 hr.

Effect of glycerol as compared with mannitol and sorbitol — The results given in Table 3 show that mannitol and sorbitol stimulate penicillin synthesis to a greater extent than glycerol in the later stages, this difference not being apparent till 14 hr. The reducing sugar in the medium throughout, in all cases, was below 0·05 per cent. The mycelial weight was higher with sorbitol and mannitol than with glycerol.

Effect of pyruvate, citrate, succinate and DL-*malate* — These compounds, involved in the citric acid cycle, were tried in concentrations of 1 per cent (wt/vol.) as the sodium salts but there was no stimulation of penicillin synthesis in these cases over the control system, except that there appeared to be a slight increase with pyruvate. Pyruvate disappeared from the medium to a small extent towards the end, whereas very little of citrate disappeared from the medium. In the preliminary experiments carried out with L-malate, the rate of disappearance was also found to be very low. It is likely that there is a permeability

TABLE 1 — ACCUMULATION OF PYRUVATE DURING INHIBITION BY ARSENITE

[*System: washed cell suspension plus glycerol* (1%) *plus PA system*; 46 *hr mycelium from fermentor* (*batch No.* 2951, *strain HA*-9) *was washed and resuspended in* 0·025*M phosphate buffer* (*p*H 7), *containing* 0·05% *PA. Initial mycelial dry weight*, 2·17%]

CONSTITUENTS ADDED	ASSAY	AT START	4 HR	8 HR	12 HR	24 HR
Glycerol	Penicillin, units/ml.	—	62·00	130·00	356·000	640·00
	Glycerol, %	1·00	0·83	0·55	0·250	0·00
	Pyruvate, mg./ml.	—	0·01	0·01	0·004	0·01
Glycerol + arsenite (0·005*M*)	Penicillin, units/ml.	—	30·00	68·00	148·000	370·00
	Glycerol, %	1·00	0·87	0·64	0·390	0·00
	Pyruvate, mg./ml.	0·01	0·87	1·08	1·030	1·06
Glycerol + arsenite (0·005*M*) + sodium acetate (0·5%)	Penicillin, units/ml.	—	28·00	50·00	109·000	380·00
	Glycerol, %	1·00	0·88	0·68	0·390	0·00
	Pyruvate, mg./ml.	0·01	0·89	0·98	1·100	1·06

barrier against these compounds, so that they do not easily get into the cell. There was a decrease in the mycelial content in all cases.

Effect of inositol — The results presented in Fig. 3 show that the stimulatory effect of 0·2, 0·7 and 1·2 per cent inositol is of the same order up to 14 hr. With 1·2 per cent (wt/vol.) inositol, it persists longer, probably because of inositol lasting longer. In the control, the mycelial weight had decreased as usual at the end of 24 hr, while there was not much change from the initial weight with inositol, 1·2 per cent inositol giving the highest weight.

Inhibitory effect of DNP, cyanide and arsenite in the PA plus inositol system — DNP, cyanide and arsenite in concentrations of 0·00006*M*, 0·0001*M* and 0·0002*M* respectively did not show any inhibitory effect. On the other hand, in concentrations of 0·0006*M*, 0·005*M* and 0·005*M* respectively they show definite inhibitory effect as seen from Fig. 4. Here again, arsenite shows almost total inhibition. There was accumulation of pyruvate to the extent of 1 mg./ml. in the medium in the case of arsenite (Table 4). The inhibitory effect of DNP and cyanide is considerable up to 14 hr, after which it disappears, probably due to the destruction of cyanide and metabolism of DNP. There is a parallelism between the rate of disapperance of inositol and penicillin production. In the case of arsenite, where there is almost total inhibition, inositol is not utilized. As regards mycelial percentage, there is an increase in the case of inositol alone; there is maximum loss when arsenite is added, and a little less with DNP. In the case of cyanide, the mycelial percentage is not altered.

Effect of inositol as compared with mannitol and dulcitol — The results given in Table 5 show that inositol is about as effective as mannitol, while dulcitol does not stimulate to the same extent. In the case of mannitol, 0·27 per cent was still present at the end of 24 hr.

Effect of inositol as compared with xylose and ribose — The results given in Table 6 show that at the end of 24 hr, xylose and ribose show greater stimulation of penicillin synthesis than inositol, xylose being better than ribose. While the pentoses disappeared fast,

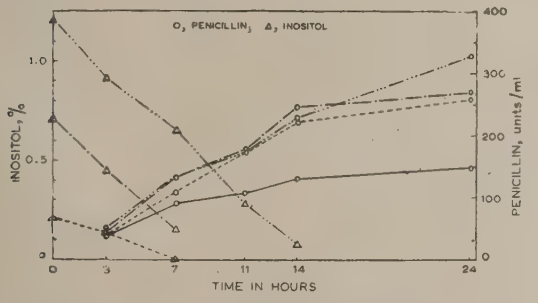

FIG. 3 — EFFECT OF INOSITOL ON THE BIOSYNTHESIS OF BENZYLPENICILLIN [Washed cells (batch No. 2113, 35 hr) were suspended in phosphate buffer with 0·05 per cent PA. ——, no carbohydrate; - - - - -, 0·2 per cent inositol; — · —, 0·7 per cent inositol; — · · · —, 1·2 per cent inositol]

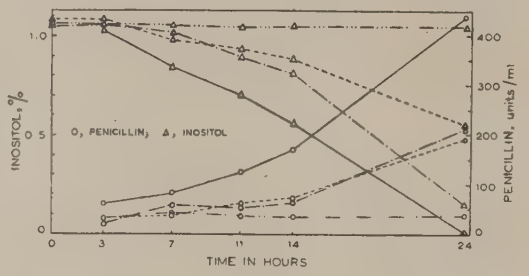

FIG. 4 — EFFECT OF INHIBITORS ON THE BIOSYNTHESIS OF PENICILLIN [Washed cells of *P. chrysogenum* (batch No. 2125, 35 hr) suspended in 0·025*M* phosphate buffer (*p*H 7) with PA (0·05 per cent) and inositol (1·0 per cent). ——, without inhibitor; - - - - -, with DNP (0·0006*M*); — · —, with cyanide (0·005*M*); — · · · —, with arsenite (0·005*M*)]

TABLE 2 — EFFECT OF GLYCEROL, GLUCOSE AND GALACTOSE

[35 *hr mycelium from fermentor (batch No.* 2012, *strain HA-6) was washed and resuspended in* 0·025*M phosphate buffer* (*p*H 7·0). *Initial mycelial dry weight,* 1·77% (*g./*100 *ml. suspension*)]

CONSTITUENTS ADDED	ASSAY	AT START	3 HR	7 HR	11 HR	14 HR	24 HR	*p*H AT 30 HR	MYCELIAL WT AT 30 HR %
PA (0·05%)	P	—	35·00	100·00	115·00	124·00	162·00	7·8	1·29
	S	0·06	0·02	0·01	0·02	0·01	0·02	—	—
PA + glycerol (1%)	P	—	32·00	130·00	230·00	347·00	517·00	7·0	1·99
	S	0·06	0·03	0·02	0·02	0·02	0·03	—	—
PA + glucose (1%)	P	—	34·00	125·00	260·00	350·00	504·00	6·5	2·05
	S	1·20	0·94	0·63	0·23	0·03	0·03	—	—
PA + galactose (1%)	P	—	38·00	120·00	280·00	341·00	509·00	7·0	1·97
	Ga	1·14	0·94	0·62	0·23	0·04	0·04	—	—

P, penicillin concentration, units/ml.; S, reducing sugar percentage (wt/vol.) calculated as glucose; Ga, galactose percentage.

TABLE 3 — EFFECT OF GLYCEROL, MANNITOL AND SORBITOL*

[34 *hr mycelium from fermentor (batch No.* 2021, *strain HA*-6) *was washed and suspended in* 0·025*M phosphate buffer* (*p*H 7·0). *Initial mycelial dry weight,* 1·78% (*g./*100 *ml. suspension*); *N content of mycelium,* 5·47%]

Constituents added	Penicillin titre μ/ml.				Mycelial wt at 30 hr %	N in mycelium %
	7 hr	11 hr	14 hr	24 hr		
PA (0·05%)	83	117	122	179	1·18	4·51
PA (0·05%) + glycerol (1%)	94	198	259	386	1·75	4·72
PA (0·05%) + mannitol (1%)	98	173	225	480	1·99	4·87
PA (0·05%) + sorbitol (1%)	103	188	267	441	1·91	4·56

*Reducing sugar was estimated at 0, 3, 7, 11, 14 and 24 hr in the filtrates and found to be below 0·05% in all cases.

TABLE 4 — ACCUMULATION OF PYRUVATE DURING INHIBITION BY ARSENITE AND DNP

[*System: washed cell suspension plus PA plus DNP.* 46 *hr mycelium from fermentor (batch No.* 2978, *strain HA*-9) *was washed and resuspended in* 0·025*M phosphate buffer* (*p*H 7) *containing* 0·05% *PA. Initial mycelial dry weight,* 2·55%]

Constituents added	Assay	At start	4 hr	8 hr	12 hr	24 hr
Inositol (1%)	Inositol, %	1·00	0·77	0·47	0·17	0·00
	Pyruvate, mg./ml.	0·00	0·00	0·00	0·00	0·00
Inositol (1%) + arsenite (0·005*M*)	Inositol, %	1·00	0·98	0·94	0·90	0·79
	Pyruvate, mg./ml.	0·00	0·90	0·96	1·06	0·97
Inositol (1%) + DNP (0·0006*M*)	Inositol, %	0·98	0·84	0·61	0·34	0·01
	Pyruvate, mg./ml.	0·00	0·03	0·05	0·03	0·02

TABLE 5 — EFFECT OF MANNITOL, DULCITOL AND INOSITOL

[35 *hr mycelium from fermentor (batch No.* 2094, *strain HA*-6) *was washed and resuspended in* 0·01*M phosphate buffer* (*p*H 7). *Initial mycelial dry weight,* 1·66%]

Constituents added	Penicillin titre μ/ml.						*p*H at 28 hr	Mycelial wt at 28 hr %
	3 hr	7 hr	11 hr	14 hr	24 hr	28 hr		
PA (0·05%)	37	105	112	106	132	169	8·0	1·24
PA + mannitol (1%)	36	123	135	153	314	409	7·1	1·54
PA + dulcitol (1%)	31	118	124	123	266	312	7·4	1·40
PA + inositol (1%)	41	150	152	129	322	308	7·2	1·50

TABLE 6 — EFFECT OF RIBOSE, XYLOSE AND INOSITOL

[40 *hr mycelium from fermentor (batch No.* 2292, *strain HA*-6) *was washed and resuspended in* 0·025*M phosphate buffer* (*p*H 7). *Initial mycelial dry weight,* 2·11%; *N content of mycelium,* 5·20%]

Constituents added	Assay	At start	3 hr	7 hr	11 hr	14 hr	24 hr	*p*H at 24 hr	Mycelial wt at 24 hr %	N content of mycelium at 24 hr %
PA (0·05%)	P	—	68·00	134·00	147·00	203·00	267·00	7·75	1·61	4·72
	S	0·02	—	—	—	—	—	—	—	—
PA + ribose (1%)	P	—	58·00	126·00	180·00	231·00	391·00	7·00	2·16	4·70
	R	1·02	0·84	0·56	0·16	0·04	0·05	—	—	—
PA + xylose (1%)	P	—	53·00	126·00	220·00	269·00	445·00	7·00	2·12	4·65
	X	0·99	0·86	0·50	0·10	0·02	0·03	—	—	—
PA + inositol (1%)	P	—	76·00	142·00	234·00	233·00	352·00	7·00	2·19	4·93
	I	1·05	1·00	0·81	0·56	0·40	0·00	—	—	—

P, penicillin concentration, units/ml.; S, reducing sugar percentage calculated as glucose; R, ribose percentage (wt/vol.); X, xylose percentage; I, inositol percentage.

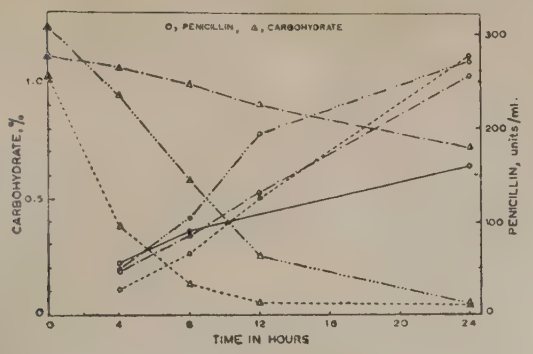

FIG. 5 — EFFECT OF DNA, GLUCURONOLACTONE AND *myo*-INOSOSE-2 ON THE BIOSYNTHESIS OF BENZYLPENICILLIN [Washed cells (batch No. 2552, 45 hr) were suspended in phosphate (0·025*M*) buffer (*p*H 7) with 0·05 per cent PA. ——, no carbon source; - - - - -, 1 per cent DHA; — · —, 1 per cent glucuronolactone; — · · · —, 1 per cent inosose. DHA, glucuronolactone and inosose were estimated by the Somogyi method]

inositol was metabolized much slower. The stimulatory effect of the pentoses becomes pronounced after 14 hr, possibly because they have to undergo some change to cause greater stimulation.

Effect of DHA, glucuronolactone and myo-inosose-2— The results presented in Fig. 5 show that all three increase the rate of penicillin synthesis. Glucuronolactone disappears very slowly and, compared to the quantity metabolized, the stimulatory effect is considerable. The mycelial weight decreases when glucuronolactone is added. In the other two cases there is a slight increase. As regards the nitrogen content of the mycelia, there is a decrease in the case of DHA and glucuronolactone, while in the case of inosose it is slight.

Discussion

The system employed in these studies for assessing the stimulatory effect of various compounds satisfies the conditions postulated as optimum by Johnson[15] for penicillin biosynthesis by *P. chrysogenum*[1,2], simulates the conditions prevailing in the fermentors in production runs, and differs in detail from those adopted by Rolinson[16], Demain[17], Halliday and Arnstein[18] and others. Determination of the mycelial percentage in the initial suspension as well as after 24 hr shows that, in the absence of sugar, there is always a decrease in the mycelial weight of about 15 to 27 per cent, probably due to autolysis of the mycelium as shown also by the rise in the *p*H of the medium. When the sugars are added, there is either not much change or only an increase of up to about 15 per cent in 24 hr. The nitrogen content of the mycelia in all cases shows a decrease at the end of 24 hr. Some nitrogenous compounds including peptides are released into the medium by the mould, one of which is penicillin. This supports the concept that penicillin is an excretory product of the mould after it reaches a certain stage of maturity and does not multiply appreciably[19,20].

In the system employed, penicillin synthesis is stimulated by carbohydrates such as glucose, galactose, mannitol, sorbitol, dulcitol, *myo*-inositol, *myo*-inosose-2, glucuronolactone, xylose, ribose, glycerol and DHA in different degrees. Such a stimulation can be caused by the supply of some precursor of penicillin which has been rate-limiting, or by providing energy for speeding up either the transport of PA into the mycelium or the various reactions connected with penicillin biosynthesis, or by the carbohydrates taking part in some way in a mechanism coupled with the biosynthesis. We have reasons to believe[2,19] that the carbohydrates, under the conditions tried, do not provide precursor units for the biosynthesis, and so we rule out the first possibility.

In the system studied, there is no appreciable synthesis of benzylpenicillin with the carbohydrate alone without PA. But with the addition of PA alone, without any carbohydrate, the synthesis proceeds for some time at a steady rate, after which it slows down due to some factor becoming rate-limiting, and this rate is increased and the synthesis prolonged by adding any of a number of carbohydrates[1,2]. The analysis of the mycelium of *P. chrysogenum* has shown that the water-soluble fraction contains a pool of free reducing sugars, amounting to about 15 per cent of the weight of the mycelium, most of which is glucose[21]. We postulate that this sugar in the pool plays a part in the biosynthesis of penicillin and is rate-limiting, and could be augmented or replenished by the carbohydrates added to the medium in the experiments. It has been shown by us[4,21] by growth experiments and analysis of the mycelia that *P. chrysogenum* is able to convert all the carbohydrates tried here into glucose to feed the sugar pool and for synthesis of the cellular polysaccharides. It is, therefore, suggested that under the conditions of the present experiments, the various carbohydrates added to the medium get converted into glucose and feed the sugar pool. Lactate, pyruvate, citrate, succinate and malate do not stimulate penicillin synthesis, and in these cases, the mycelial percentages also show no differences from the controls without sugar. It may be that these compounds cannot enter the cell due to permeability barriers.

The metabolism of glucose in the water-soluble sugar pool of the mycelium is postulated here to be involved in the condensation of the precursor units to yield penicillin as indicated below. The biosynthesis of benzylpenicillin from phenylacetic acid and

the two amino acids, cysteine and valine, involves the formation of two or three peptide-like bonds and a dehydrogenation[19]. Since the peptide bond is known to be formed by the activation of the carboxyl groups of the amino acids by ATP[22-24] for the continued synthesis of penicillin, an uninterrupted supply of ATP molecules is essential. This is supported by the observation that DNP, cyanide and arsenite inhibit the biosynthesis of penicillin. The formation of ATP from glucose and also other sugars is possible by two mechanisms, anaerobic substrate-linked phosphorylation and oxidative phosphorylation[25]. Since aeration is absolutely essential for penicillin biosynthesis which is also affected by DNP, known to uncouple oxidative phosphorylation, it is tempting to suggest that ATP molecules are produced by the latter process which involves the terminal electron transport chain. The ineffectiveness of the citric acid cycle intermediates is probably due to the difficulty of their getting into the cells from the medium. The dehydrogenation involved in the biosynthesis may well be coupled with the flavoproteins or the cytochromes of the electron transport chain. Since penicillin biosynthesis is inhibited by cyanide, linking to the cytochromes may be more probable and the presence of cytochromes has been detected in *P. chrysogenum* by Sih *et al.*[26].

The inhibitors studied affect the metabolism of the sugars added to the medium, and there is a correlation between the rate of disappearance of carbohydrates and penicillin synthesis. In the case of arsenite, where there was complete inhibition, very little of the sugar added was used up; the accumulation of pyruvate in the medium from glycerol and inositol has also been noted. This is easy to understand since arsenite is known to affect oxidative decarboxylation of pyruvate.

Acknowledgement

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Toxicity Studies on Psoralen, Isopsoralen & Imperatorin in Albino Rats

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The toxic effects of psoralen, imperatorin, and a mixture (1 : 1) of psoralen and isopsoralen, employed in the treatment of leucoderma, have been investigated employing albino rats as the experimental animals. The drugs have been administered at a daily dosage level of 2·5 mg. per 75 g. body weight of rat for 60 days. No gain in weight has been observed in the case of the ten rats administered psoralen and isopsoralen mixture, but the weight of spleen has been found to increase two-fold. Hyalinization of the lung alveoli has been observed in one animal, and haemorrhage from the mouth, nose and anus in two other animals; in the latter, hypertrophy of the bronchial lining has also been observed. Of the ten rats receiving psoralen alone, haemorrhage from the mouth, nose and anus has also been observed in one rat. Psoralen, imperatorin and psoralen-isopsoralen mixture have also been found to produce hypertrophy of liver, kidney and spleen.

AMMI majus Linn. has been widely used in Egypt in the treatment of leucoderma[1,2]. The drug (*c.* 4-12 g.) was administered orally and the patients were exposed to sunlight. This is reported to bring about repigmentation of the white patches, but according to Lerner *et al.*[2], the drug causes certain undesirable side effects, such as severe vomiting, diarrhoea, coma, abdominal pain, nephritis, hepatitis, cirrhosis of the liver and exfoliative dermatitis.

Fahmy and Abu-Shady[3] isolated three crystalline principles, all furocoumarin derivatives, from the above plant, viz. 8-methoxypsoralen (xanthotoxin), 5-methoxypsoralen (bergapten) and 8-isoamylene oxypsoralen (imperatorin). Lerner *et al.*[2] found that a 15 months old child could take 10 mg. of 8-methoxypsoralen daily for months without adverse effects. An adult was reported to tolerate a daily dose of 50 mg. while another could not tolerate a dose exceeding 20 mg. per day without suffering from nausea, epigastric distress, increased nervous tension and diarrhoea. These workers, however, have recommended a dosage up to 50 mg. daily.

El Mofty[4] found that in the case of mice, the M.L.D. for 8-methoxypsoralen and 8-isoamylene oxypsoralen was 300 mg./kg. and 330 mg./kg. respectively. He, however, did not study the effect of the drugs given in small doses over prolonged periods. Elwi[5] has observed that in the case of guinea-pigs, the M.L.D. for 8-methoxypsoralen was 400 mg./kg. and for 8-isoamylene oxypsoralen 800 mg./kg. With the administration of sublethal doses of 8-methoxypsoralen (200-350 mg./kg.) the animals exhibited cloudy swelling, fatty degeneration and acute haemorrhagic necrosis of the liver in 30 hr. The kidneys were severely congested and haematuria was present. With doses of 300 mg./kg., the adrenals showed congestion and lipoid degeneration, and with a daily dose of 1 and 2 mg./kg., administered for a period of 5 months, there was necrosis of the liver in some animals. At the same dose level, 8-isoamylene oxypsoralen caused only some cloudy swelling of the liver.

In this Institute, a mixture of psoralen (6,7-furocoumarin) and isopsoralen (7,8-furocoumarin) has been administered orally to leucoderma patients in doses of 50 mg. for prolonged periods, but no systematic toxicity studies of this treatment have been undertaken. In this paper, the results of such a study employing albino rats as the experimental animals are presented.

Experimental procedure

Albino rats as bred in this Institute and weighing about 75 g. were used. The animals were divided in groups of five each. Two groups of 10 animals each received the drug and two more groups of 10 animals each to which no drug was administered served as

controls. The drugs to be administered were well ground with twice their weights of gum tragacanth and a drop or two of Tween '80', and mixed with water to contain 5 mg. of the drug in each ml. of the solution. Each experimental animal received orally 0·5 ml. of the drug every day, while the controls received the tragacanth solution without drugs. The animals were weighed weekly and any changes in the appearance of the fur, food intake or any other abnormality for a total period of 10 weeks were noted. They were then killed with chloroform and examined. In the case of animals administered psoralen and isopsoralen mixture, however, the animals registered an increase in weight during the course of the experiment but the weight came down to the initial value or less at the end. The animals were killed after 40 days. Post-mortem examinations were done and livers, kidneys and spleens were weighed and, along with other tissues, put in 10 per cent neutral formol saline. The tissues were examined histologically after cutting sections from paraffin blocks and staining with haematoxylin and eosin.

Results and discussion

Rats receiving psoralen developed a certain degree of furred coat after 18-19 days of treatment. One rat exhibited bending of the head to the left and also showed circular movement; this animal died two days later. Four other rats in this group were sacrificed along with five animals from the control group and examined. In the psoralen group, post-mortem examination revealed nothing abnormal beyond certain amount of congestion and enlargement of the livers. After 68 days the remaining 5 psoralen-treated and 5 control rats were killed. Post-mortem examination of psoralen-treated rats revealed congestion and enlargement of liver.

In the rats treated with psoralen and isopsoralen mixture, bending of the head to one side was observed in two rats after 17 and 24 days respectively; the parotid glands of these rats were found enlarged. Two other rats in this series were killed after 40 days and examined, since these rats exhibited a temporary small increase in weight during the experiment and which returned to the original value at the end.

One rat died on the 35th day with bleeding from the mouth, nose and anus. The livers of these animals were somewhat congested and enlarged. In the animal which died with bleeding from the mouth, nose and anus, the lungs were found to be haemorrhagic.

Rats treated with imperatorin appeared to be growing normally. These were killed along with controls after 60 days. The livers of these animals were, however, found to be somewhat congested and enlarged.

Histological examination of the liver, spleen, heart, lungs, kidney and suprarenals showed that the lungs of rats receiving psoralen and isopsoralen mixture had undergone hypertrophy of the cells lining bronchi in a few cases and in two cases certain degree of hyalinization of the alveoli was also observed. No other significant damage was observed in other tissues. Necrosis of the liver reported by Lerner *et al.* was not observed.

El Mofty[4] and also Lerner *et al.*[2] consider that 8-methoxypsoralen administered at a daily dose level of 50 mg. is safe for human adults. On considerations of surface area, a rat weighing 100 g. should be able to tolerate a daily dosage of about 1·9 mg. of 8-methoxypsoralen. In the present study, a larger convenient daily dose of 2·5 mg. per 75 g. body weight of rat was administered orally. It would appear from the results obtained in this study that psoralen has no effect on the growth of rats (Table 1). A two-fold increase in the weight of spleen in relation to body weight has been observed and this is a striking feature. While with psoralen and imperatorin, and the mixture of psoralen and isopsoralen there was about 20 per cent increase in the weight of liver in relation to the body weight, increase in the weight of kidney was not so striking; it was, however, prominent in the case of rats

TABLE 1 — WEIGHT OF DIFFERENT ORGANS IN RELATION TO BODY WEIGHT OF RATS TREATED WITH DIFFERENT DRUGS

(Ten animals were employed in tests for each drug and 30 animals as controls)

Drug	Initial wt of rat g.	S.D.	Final wt of rat g.	S.D.	Liver wt as % body wt	S.D.	Kidney wt as % body wt	S.D.	Spleen wt as % body wt	S.D.
Psoralen	75·0	±1·78	170·0	±4·20	5·0	±0·45	0·930	±0·010	0·390	±0·045
Psoralen and isopsoralen	90·0	±1·45	87·4	±1·89	5·0	±0·36	1·090	±0·050	0·640	±0·027
Imperatorin	69·6	±2·16	138·8	±4·12	4·9	±0·38	0·902	±0·019	0·309	±0·008
Control	74·4	±2·61	143·4	±3·50	4·1	±0·32	0·870	±0·014	0·350	±0·041

administered psoralen-isopsoralen mixture. It would, therefore, appear that psoralen and isopsoralen mixture in equal parts is toxic for rats while psoralen and imperatorin administered singly can be considered safe at the dose indicated.

Acknowledgement

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Effect of Cadmium Chloride on Gonadotrophin Content of the Pituitary of Male & Female Rats

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A single subcutaneous injection of cadmium chloride (1 mg./100 g. body weight) to adult male rats causes a prompt decline in pituitary FSH level which persists up to 30 days. The LH concentration, on the other hand, shows almost a linear rise during this period. The pituitary FSH and LH of young female rats treated similarly with cadmium chloride show different type of alterations. The immediate response of FSH is a sharp rise which is followed by a gradual return to normal level within 7 days. In contrast, LH concentration registers a drastic reduction to begin with but attains normal picture within 7 days. The bearing of these findings on overall mechanism of action of cadmium on gonads is discussed.

THE unusual effects of cadmium chloride on the gonads of male and female rats have been reported[1-3]. There is, however, a basic difference between the sexes with respect to the nature of reactions to cadmium; in males the destruction of seminiferous epithelium is prompt and irreversible, whereas atresia of the follicles in the female is more phasic but evanescent. The other interesting feature in males is the regeneration of Leydig cells after initial mass degeneration of the interstitial elements and the gradual return of endocrine activities of the testes[1,2].

Any influence of cadmium on the response of the ovary to exogenous gonadotrophin has been investigated[3]. An inhibitory effect of a more quantitative nature is noticed up to 120 hr after administration of the metal but disappears at 360 hr when the ovary responds fully to gonadotrophin. It is also significant that the severity of cadmium effect on the ovary is considerably reduced by gonadotrophin.

The above findings, together with the general nature of gonadal changes, suggest a disturbance of pituitary gonadotrophic activity. To test one aspect of this possibility, an attempt has been made to estimate gonadotrophin content (FSH and LH) of the pituitary of male and female rats at different time intervals after administration of cadmium chloride and the results obtained from this study are presented in this communication.

Experimental procedure

Colony-bred male (100-150 g.) and female (80-120 g.) albino rats of this Institute were injected with cadmium chloride by the subcutaneous route (1 mg./100 g. body weight at the interscapular region, single injection). The males were sacrificed at 0, 2, 7, 14, 30 and 90 days after cadmium administration. Comparable period of observation was, however, brief in the case of females (0, 1, 2 and 7 days after cadmium

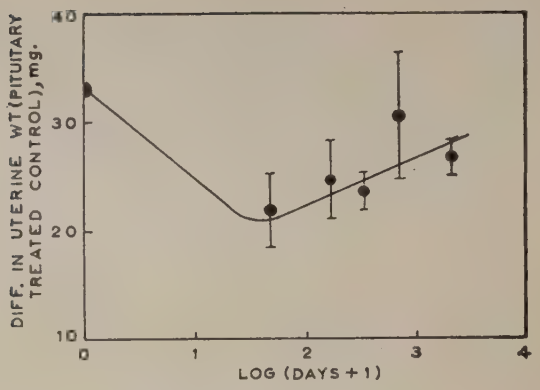

FIG. 1 — CHANGES IN UTERINE WEIGHT OF MICE AT DIFFERENT TIME INTERVALS AFTER INJECTION OF PITUITARY FROM CADMIUM CHLORIDE-TREATED MALE RATS

injection) because of the characteristic reversibility of ovarian changes within a week.

Pituitary from donor rats was dissected out on autopsy, weighed to the nearest 0·1 mg. in a Roller-Smith balance and then homogenized with physiological saline solution in such a manner that 1 ml. of the homogenate was equivalent to 1 mg. of the gland. Pituitary from 15 to 20 rats were pooled and processed for assay at each time interval. Testis and ovary of donor rats were fixed in Bouin's fluid and serial paraffin sections were stained with haematoxylin and eosin.

FSH content of the pituitary was determined by the procedure of Levin and Tyndale[4]. Albino mice of a Swiss strain (6-8 g.) maintained in the Institute colony were injected subcutaneously with 1 ml. of the pituitary homogenate daily for 3 days. The animals were sacrificed 24 hr after the final injection. The uterus was dissected out, pressed between pieces of filter paper to remove the fluid and then weighed to the nearest 0·1 mg. on a Roller-Smith balance.

Pituitary LH content was assayed by the method of Loraine[5]. Male albino rats of the Institute colony (30-45 g.) were injected subcutaneously with 1 ml. of the pituitary homogenate daily for 3 days. The animals were killed 24 hr subsequent to the final treatment. The prostate (both ventral and dorso-lateral) was dissected out and weighed as above.

The results of assay are expressed in terms of the difference between the control (injected with saline) and pituitary-treated test animals with respect to uterine and prostatic weights.

Results

Effect of cadmium chloride on FSH content of the pituitary of male and female rats

Male rats — Two days after cadmium chloride administration pituitary FSH content registered a significant fall as indicated by the low uterine weight in comparison to 0 day group ($P < 0{\cdot}01$; Table 1, Fig. 1). Such diminished FSH level persisted almost uniformly during the rest of the experimental period except at 30 days when it tended to rise somewhat (0 versus 7, 14 and 90 days; $P < 0{\cdot}05$, 0·001 and 0·01 respectively).

Female rats — In contrast to males, the FSH content of the pituitary of female rats showed a sharp rise on the day subsequent to cadmium chloride administration. But after 2 days there was a little decline in FSH concentration and by 7 days the initial level (0 day) was reached (Fig. 2). However, the difference in uterine weight between 0, 1 and 2 days groups was not statistically significant because of considerable variations (Table 1).

Effect of cadmium chloride on LH content of the pituitary of male and female rats

Male rats — LH content of the pituitary of cadmium chloride-treated male rats rose almost in a linear fashion up to 30 days but dropped at 90 days (Fig. 3). However, at 14 days a downward trend of LH level was noticed which was reversed during the subsequent 30 days. It will also be evident from Table 2 that the variations in prostatic weight were wide and as such the mean values at various time intervals did not reveal any statistically significant difference.

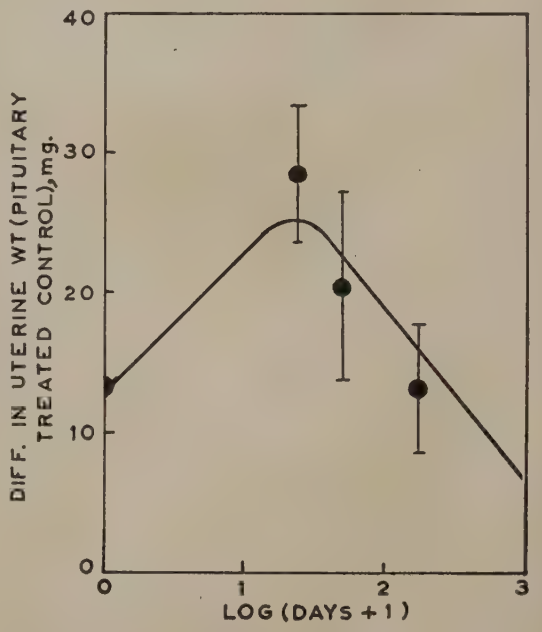

FIG. 2 — CHANGES IN UTERINE WEIGHT OF MICE AT DIFFERENT TIME INTERVALS AFTER INJECTION OF PITUITARY FROM CADMIUM CHLORIDE-TREATED FEMALE RATS

TABLE 1 — UTERINE WEIGHT OF MICE AT DIFFERENT TIME INTERVALS AFTER INJECTION OF PITUITARY FROM CADMIUM CHLORIDE-TREATED MALE AND FEMALE RATS

Treatment	Male: mean uterine wt (*mg.*) with S.E.						Female: mean uterine wt (*mg.*) with S.E.			
	0 day	2 days	7 days	14 days	30 days	90 days	0 day	1 day	2 days	7 days
Control (saline)	10·94±0·55 (8)*	9·44±0·45 (5)	7·90±0·77 (6)	5·33±0·13 (6)	8·41±0·47 (6)	7·45±1·26 (5)	16·58±4·86 (11)	17·00±3·21 (3)	14·33±1·79 (3)	17·68±3·19 (5)
Pituitary	44·33±0·40 (6)	31·43±3·42 (7)	32·79±1·29 (7)	29·03±1·74 (7)	38·90±5·81 (6)	34·16±0·95 (5)	30·00±8·10 (5)	45·87±3·16 (4)	34·60±6·10 (5)	31·10±3·59 (4)
Difference (pituitary minus control)	33·39±0·68	21·99±3·45	24·89±3·65	23·70±1·75	30·49±5·83	26·71±1·58	13·42±9·45	28·87±4·50	20·27±6·35	13·42±4·80

*Figures in parentheses indicate number of animals.

TABLE 2 — PROSTATIC WEIGHT OF RATS AT DIFFERENT TIME INTERVALS AFTER ADMINISTRATION OF PITUITARY FROM CADMIUM CHLORIDE-TREATED MALE AND FEMALE RATS

Treatment	Male: mean prostatic wt (*mg./100 g. body wt*) with S.E.						Female: mean prostatic wt (*mg./100 g. body wt*) with S.E.			
	0 day	2 days	7 days	14 days	30 days	90 days	0 day	1 day	2 days	7 days
Control (saline)	95·70±6·05 (7)*	121·68±6·87 (7)	88·80±9·73 (6)	89·56±4·39 (6)	112·58±7·67 (7)	82·29±10·16 (5)	88·98±4·14 (11)	95·48±7·41 (3)	94·28±6·42 (3)	80·79±6·82 (4)
Pituitary	113·11±3·29 (7)	159·50±10·74 (6)	133·52±6·98 (7)	114·4±10·90 (7)	155·51±9·61 (6)	106·96±15·62 (5)	91·76±5·25 (5)	95·63±6·26 (3)	93·13±4·17 (5)	83·79±6·41 (5)
Difference (pituitary minus control)	17·41±6·89	37·82±12·75	41·43±11·97	24·85±11·75	42·93±12·30	24·67±18·63	2·78±6·68	0·15±9·67	−1·15±7·66	3·00±9·36

*Figures in parentheses indicate number of animals.

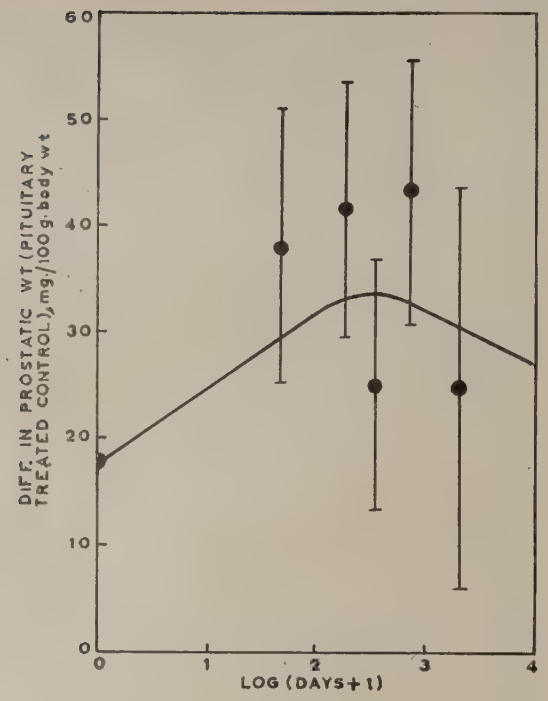

FIG. 3 — CHANGES IN PROSTATIC WEIGHT AT DIFFERENT TIME INTERVALS AFTER INJECTION OF PITUITARY FROM CADMIUM CHLORIDE-TREATED MALE RATS

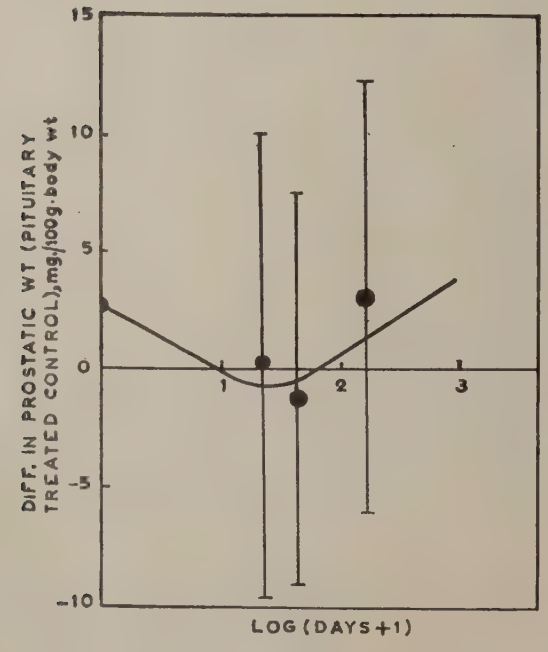

FIG. 4 — CHANGES IN PROSTATIC WEIGHT AT DIFFERENT TIME INTERVALS AFTER INJECTION OF PITUITARY FROM CADMIUM CHLORIDE-TREATED FEMALE RATS

Female rats — The pattern of change in pituitary LH level in females was different from that in males. Thus, the concentration of this hormone declined drastically during the first two days after cadmium chloride administration. So much so that at 2 days LH content of the pituitary fell below the basal level and minus values were actually obtained. However, by 7 days the concentration of the hormone returned to the relatively high 0 day level (Table 2; Fig. 4). Here again, the variations in prostatic weight did not lead to statistically significant difference between the groups (Table 2).

Effect of cadmium chloride on pituitary weight of male and female rats

Male rats — Pituitary weight of male rats did not change appreciably up to 2 days but at 7 days an increase was noticed. This situation persisted for 14 days, after which there was a progressive decline (Table 3; Fig. 5). It was to be noted that the pituitary weight at 30 and 90 days was significantly greater than that at 0 day ($P < 0.02$).

Female rats — In the female the changes in pituitary weight followed a somewhat different pattern. This was indicated by a slight fall in the weight of the gland on the day following cadmium chloride administration and a progressive rise during the rest of the experimental period (Table 3; Fig. 5). The pituitary weight at 2 and 7 days was significantly greater than that of 0 and 1 day groups ($P < 0.02$). Similarly, the weight of the gland at 2 days was greater than that at 1 day ($P < 0.01$); the difference between 2 and 7 days groups was also statistically significant ($P < 0.001$).

TABLE 3 — PITUITARY WT OF MALE AND FEMALE RATS AFTER $CdCl_2$ ADMINISTRATION

TIME AFTER $CdCl_2$ INJECTION *days*	MEAN PITUITARY WT (*mg./100 g. body wt*) WITH S.E.	
	Male	Female
0	3·49±0·21 (15)*	3·83±0·11 (15)
1	—	3·42±0·22 (15)
2	3·14±0·20 (20)	4·20±0·13 (15)
7	4·44±0·38 (20)	5·41±0·13 (20)
14	4·44±0·21 (14)	—
30	4·36±0·23 (15)	—
90	4·01±0·12 (15)	—

*Figures in parentheses indicate number of animals.

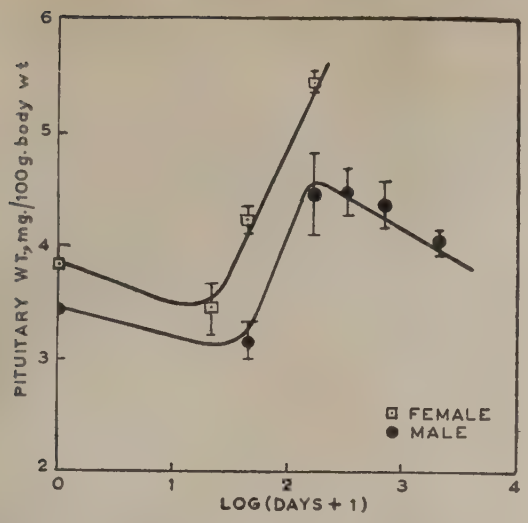

FIG. 5 — CHANGES IN PITUITARY WEIGHT OF MALE AND FEMALE RATS AT DIFFERENT TIME INTERVALS AFTER ADMINISTRATION OF CADMIUM CHLORIDE

Discussion

The results obtained in the present study indicate that the pituitary FSH and LH pattern at different periods is in keeping with the nature of response of the gonads to cadmium. The permanent destruction of the seminiferous epithelium and the dramatic recovery of the interstitium in males[2] are reflected in the changes in pituitary FSH and LH. Similarly, any alterations in the concentration of these gonadotrophins in the female are consonant with the evanescent nature of ovarian response[3].

An interesting difference is, however, noticed between the sexes when details are considered. In cadmium-treated males a low pituitary FSH level persists almost throughout the observational period. In females, on the other hand, the initial reaction to cadmium is a sharp rise in FSH concentration followed by a gradual return to normal level. These facts suggest that even with respect to its effect on the factor trophic to the germinal portion of the gonads (FSH), cadmium shows sex dichotomic properties. In all probability, this reflects the nature of contribution of the pituitary to the intricate mechanisms involved in such unusual effects of cadmium on the gonads. In males it is evident that the influence on cells responsible for the elaboration of FSH is of an irreversible nature which results in its (FSH) production (and possibly also release) at a continuously low level. Nevertheless, it is doubtful whether such low level of FSH alone could produce degenerative changes of the nature and magnitude seen in the seminiferous epithelium of cadmium-treated rats[2]. It could of course be argued that in addition to its effect at the level of the pituitary, cadmium blocks the action of FSH on testis itself and thereby creates a state of 'chemical hypophysectomy'. But even in surgically hypophysectomized rats spermatogenia, sertoli cells and spermatocytes are observed as late as 28 days after the operation; only the terminal stages of spermatogenesis are absent[6]. In contrast, the seminiferous tubules of cadmium-treated rats become transformed into a morass of dead cellular debris with no sign of survival of even a single element of the spermatogenic series[2]. Such a critical appraisal of the situation, therefore, emphasizes once again that the inhibition of pituitary FSH is but one of the aspects of a more complicated mechanism involved in the destruction of seminiferous epithelium by cadmium.

In female rats, on the other hand, the course of events suggests a more direct participation of pituitary FSH in mass atresia of the follicles induced by cadmium. The follicular destruction starts as early as 6 hr and by 2 days the process is complete[3]. It is possible that the sharp rise in pituitary FSH at 24 hr indicates its storage rather than release. This together with some loss of sensitivity of the ovary itself to FSH[3] is perhaps responsible for such destruction of the follicles. The subsequent events seemed to support this viewpoint. Thus, 2 days after cadmium administration FSH content of the pituitary commences to decline and by 7 days the normal level is reached. In all probability this progressive diminution of FSH indicates its release and the initial rise mostly storage (cf. males). It is significant that signs of differentiation of the surviving primordial ovocytes to new follicles are noticed after 2 days and at 7 days the process is complete. Moreover, any loss of sensitivity of the ovary to FSH is found to be relatively mild at a time (2 days)[3] which coincides with the commencement of release of FSH stored in the pituitary. All these arguments suggest that an initial storage in the pituitary with little discharge followed by a release of stored FSH are responsible for such actute destruction of the existing follicles and the eventual differentiation of the surviving primordial ovocytes into a new crop of follicles. It may, however, be mentioned that the simulacrum of such a mechanism of action of cadmium involving pituitary FSH has been observed in the previous study[3].

A somewhat similar difference between the sexes is noticed with regard to pituitary LH. Thus, in the males LH concentration rises almost in a linear fashion up to 90 days whereas in females an initial decline lasting for 2 days is followed by a return to normal level. It is significant that in males a down-

ward trend of LH is observed around 14 days which is again reversed during the ensuing 30 days. To what extent such changes in pituitary LH influence the nature of events in the gonads merits a critical consideration. In males cadmium causes total destruction of the interstitial elements to begin with, so much so that the entire interstitium is replaced by an eosinophilic debris of dead cellular elements. Commensurate with this, clear castration manifestations are seen in the accessory genital organs[1]. This state of affairs continues up to 10 days after which the interstitium showed signs of recovery as indicated by the presence of Leydig cells[2]. The net outcome is a gradual return of endocrine functions and the disappearance of castration changes in the genital accessories. Here again, the magnitude of initial damage to the interstitium could not be ascribed solely to the changes in LH pattern. Even if it is assumed that the characteristic linear increase in LH up to 14 days reflects either compensatory outpouring due to castration phenomena or storage with complete stoppage of release, the morphologic nature of damage is so profound that it tends to defy explanation on any of these simple grounds. Visualization of a desensitizing effect of cadmium on the testes by analogy with ovary does not quite solve the conundrum. Surgical removal of the pituitary no doubt causes atrophy of the Leydig cells[6] but never damage of such unusual nature. All these suggest that cadmium exerts its initial effect on the interstitium through a mechanism of which LH is a part. On the other hand, it seems that LH plays a more meaningful role in the recovery of the interstitial elements because the characteristic decline at 14 days might be concerned with the initiation of synthetic activities in the Leydig cells which make their first appearance at about this time[2]. Similarly, the subsequent rise in LH level is perhaps a sign of heightened pituitary function to complete the recovery of the interstitium and to ensure normal secretory activity of the Leydig cells.

As with FSH, the changes in LH pattern in females could be correlated with the nature of events in the ovarian stroma. The two major changes induced by cadmium are massive luteinization and destruction of stromal cells. The acuteness of these processes are unique because by 2 days they are complete but by 7 days they disappear and the ovary presents normal features[3]. The drastic reduction in pituitary LH during the first 2 days suggests a rapid flushing out of the hormone to bring about such bulky luteinization. Further, it is possible that the diversion of LH action by cadmium to one particular direction (luteinization) deprives most of the stromal cells of their normal quantum of this trophic factor and as a result they could not survive. An interference with the responsiveness of these cells to LH by cadmium[3] is also suggestive in this connection. On the basis of similar grounds, it could be envisaged that gradual return of normal LH pattern after 2 days is perhaps associated with the recovery of the stroma from toxic effects of cadmium.

Acknowledgement

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Letters to the Editor

EFFECT OF AUREOMYCIN (CHLORTETRACYCLINE) & AUREOMYCIN IN 5 PER CENT SODIUM CHLORIDE SOLUTION ON THE KEEPING QUALITY OF FRESH WATER FISH FILLETS

Aureomycin (chlortetracycline) in a concentration of 5 p.p.m. in 5 per cent sodium chloride solution has not been found to enhance the keeping quality of fresh water fish fillets as in the case of marine fish fillets. This is attributed to the stimulation of growth of certain types of fresh water bacteria in the presence of low concentrations of salt.

EXTENSIVE WORK HAS BEEN DONE IN OTHER COUNTRIES[1-3] to show that the storage life of marine fish fillets can be extended more than 300 per cent by dipping them for short time in 5 per cent brine containing 5 p.p.m. aureomycin (chlortetracycline) solution. However, there are no reports in literature on the preservative effect of the above treatment on fresh water fish fillets. Therefore, work was undertaken in this Institute to investigate the comparative keeping quality of fresh water fish fillets treated with 5 p.p.m. aureomycin in water and 5 p.p.m. aureomycin in 5 per cent A.R. sodium chloride solution.

Fresh water fish *Avalu* (*Ophicephalus* species), *Kooralu* (*Barbus dubius*) and *Korva* (*Ophicephalus* sp.) were caught in the Cauvery river and surrounding tanks of Mysore and brought to the laboratory immediately packed in ice. They were cleaned and the fillets were prepared under strict hygienic conditions. The fillets were then given one hour dip treatment in the following solutions: (i) 5 per cent A.R. sodium chloride solution, (ii) 5 p.p.m. aureomycin solution, and (iii) 5 per cent A.R. sodium chloride solution containing 5 p.p.m. aureomycin. After treatment, the fillets were drained for 2 min., enclosed in alkathene bags and stored under refrigerated condition (35-39°F.). The quality of the fillets was judged at intervals organoleptically and also by determining their total volatile base (T.V.B.) and bacterial count as described in the previous paper[4].

Organoleptic evaluation and chemical analyses of the stored fillets showed that fillets treated with 5 per cent A.R. sodium chloride containing 5 p.p.m. aureomycin were in no way superior to those treated with aureomycin alone (Figs. 1 and 2). *Avalu* fillets treated with salt plus aureomycin developed sour odour on the 30th day of storage (T.V.B., 17·1 mg. and bacterial load, 80 million) whereas the fillets treated with aureomycin alone were quite fresh (T.V.B., 12·8 mg. and bacterial load, 50 million). The

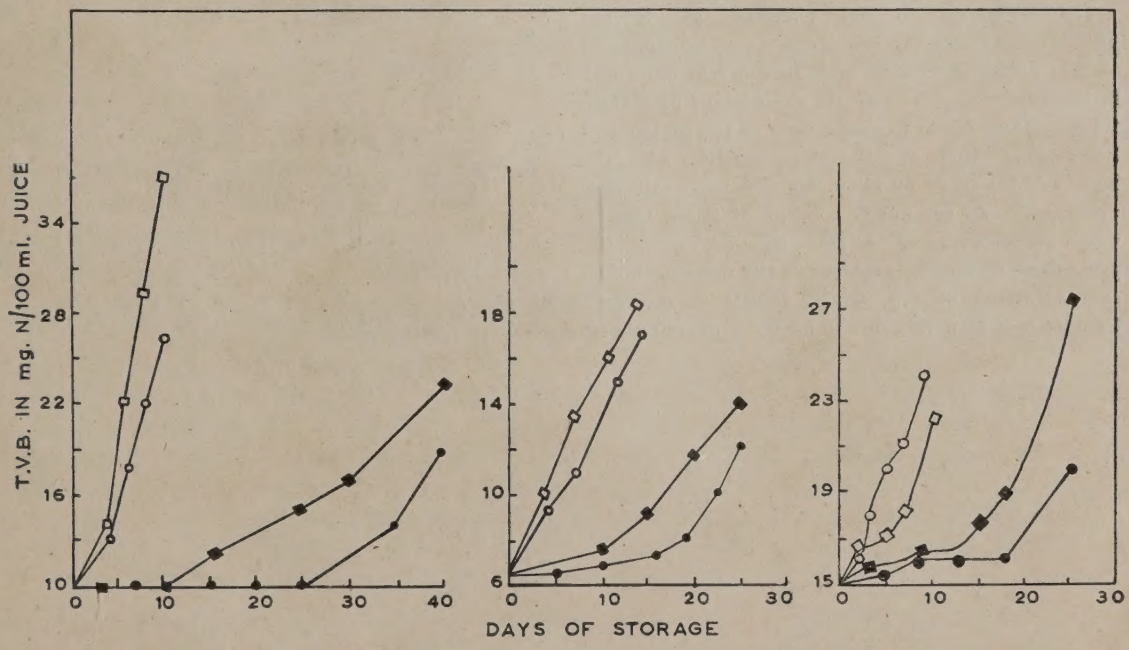

FIG. 1 — EFFECT OF DIFFERENT TREATMENTS ON THE TOTAL VOLATILE BASE CONTENT OF FILLETS DURING STORAGE [□—□, 5 per cent A.R. NaCl; ■—■, 5 per cent A.R. NaCl + 5 p.p.m. C.T.C.; ○—○, untreated; ●—●, 5 p.p.m. C.T.C.]

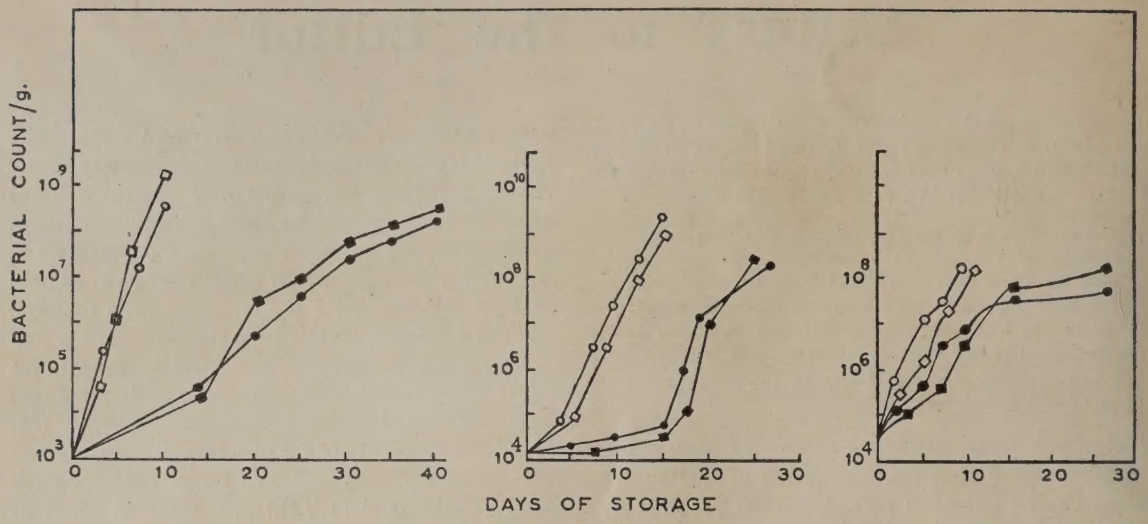

FIG. 2 — EFFECT OF DIFFERENT TREATMENTS ON THE BACTERIAL LOAD OF FILLETS DURING STORAGE [□—□, 5 per cent A.R. NaCl; ■—■, 5 per cent A.R. NaCl + 5 p.p.m. C.T.C.; ○—○, untreated; ●—●, 5 p.p.m. C.T.C.]

results with *Korva* fillets treated in the above manner were similar to those obtained with *Avalu* fillets. Further, in the case of *Kooralu* fillets, those treated with salt plus aureomycin became inedible much earlier than those treated with aureomycin alone.

It appears from the above results that treatment with 5 p.p.m. aureomycin in 5 per cent salt solution does not enhance the keeping quality of fresh water fish fillets as in the case of marine fish fillets.

The comparatively high bacterial load of the fillets treated with salt plus aureomycin as compared to that of fillets treated with aureomycin only may be attributed to the growth stimulation of certain types of fresh water bacterial flora in the presence of low concentrations of salt (1-5 per cent)[5]. Also, the high T.V.B. value of fillets treated with salt plus aureomycin as compared to that of fillets treated with aureomycin alone may also be due to the proteolysis of muscle proteins in the presence of low concentrations of salt which results in increased formation of ammonia due to bacterial deamination[6].

Further work is in progress.

The authors express their thanks to Dr A. Sreenivasan, Deputy Director, and Dr V. Subrahmanyan, Director, for their keen interest in the work.

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14 *April* 1960

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